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THE DIPLODINA DISEASE OF DELPHINIUM[1]

THOMAS LASKARIS[2]

(Accepted for publication October 14, 1949)

This fungus disease of perennial delphinium was first described by the writer in a preliminary note in which the disease was attributed to *Phoma* sp. (4). Further study of the causal organism revealed that it should be referred to the genus *Diplodina* (5). The fungus is designated *Diplodina delphinii* sp. nov. and is described in this paper.

The known distribution of the disease is limited to the areas in which it has been observed by the writer. These include Ridgefield, Connecticut, where the disease was first seen, Palisades, New Jersey, and the grounds of the New York Botanical Garden in New York City. There is no previous report of this disease in the United States or abroad, although it is probable that the disease has been confused with others in the past.

SUSCEPTIBILITY

At the New York Botanical Garden natural infection with *Diplodina* was observed on several closely related varieties or strains of *Delphinium elatum* and on many of the commercial hybrid varieties. *D. consolida* L., *D. ajacis* L., *D. grandiflorum* L., and *D. cardinale* Hook., although grown in considerable numbers, failed to show evidence of natural infection. The fungus has been isolated from stems of *Delphinium zalil* Aitch, stems and decaying crowns of Ruy's Pink Sensation (*D. nudicaule* T. and G. × *D. elatum* L.), *D. tatsienense* Franchet × *D. elatum* L., and *D. cultorum* Voss. var. Belladonna × *D. elatum* L., hybrids all growing in the plantings of a Connecticut grower.

SYMPTOMATOLOGY

The disease appears as cankers or local stem lesions, as leaf spots, as shoot or petiole soft rot, as inflorescence and seed-pod blight, and most often as a crown and root rot.

On the stem, localized necrotic lesions are brown to black and variable in size and shape. They may involve an area 3 cm. long or more, and simulate a canker. The pycnidia of *Diplodina*, which are embedded beneath the epidermal layer of the host, appear as small pin-point dots concentrically arranged over the surface of the infected area. The lesions may occur on

[1] Extract of portion of thesis, "Studies on delphinium crown rot" (1941), submitted by the writer in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota.

The investigation was supported by funds of the American Delphinium Society. Special thanks are due to Mr. Edward Steichen, President of the society, for his continued interest, close cooperation, and constant encouragement throughout the work.

[2] The writer wishes to express his appreciation of the advice and encouragement of Dr. B. O. Dodge of the New York Botanical Garden. Dr. W. J. Robbins, Director of the Garden, kindly made available the many facilities of that institution.

any part of the stem although they are most common on the lower portions. Pycnidia of the fungus may also be found in or bordering on the cracks or splits made by the stem in its normal seasonal growth. The fungus readily becomes established on these natural wounds, although it is not wholly dependent on them for entrance into the host. When mature pycnidia of the fungus are moistened, large numbers of spores ooze out in long twisting coils or cirri. The spores are held together in a gelatinous matrix which is soon dissolved by water, thereby releasing the spores for dissemination to other parts of the same plant or to other plants.

Petiole symptoms vary, depending on the succulence of the tissue at the time of infection. Diseased tissues of young green petioles appear brown and water-soaked as if affected with a soft rot; the fungus travels rapidly down the petiole, and a brown water-soaked area soon forms around the base. It is not uncommon to find the base of almost every petiole of an individual stem infected. In less succulent tissue the fungus is confined to local lesions and the invaded area is usually black. Pycnidia may develop on the infected tissue, especially on lesions formed at the point of attachment of the petiole and the leaf blade.

Following periods of wet weather, the fungus attacks the young tender basal shoots arising from the crown, producing a rapid, brown to black, watery soft rot similar to the petiole rot described. Such rots occur throughout the season when moist conditions prevail. In the spring, basal shoots of plants in an advanced stage of crown decay frequently wilt suddenly.

Symptoms on the leaves are in many respects indistinguishable from those ascribed to the bacterium *Phytomonas delphinii* (1). The *Diplodina* is commonly associated with tarry black spot lesions on the upper leaf surface, and light brown lesions on the lower surface.

Crown and root symptoms do not differ markedly from those caused by other crown-rot organisms. A black or dark brown discoloration of the vascular tissue characterizes the Diplodina disease, in contrast to the brown discoloration associated with *Rhizoctonia* and *Fusarium* crown rot. In an advanced stage of the disease, the invaded cortical tissues undergo a general necrosis, evident as a rather soft, moist black rot. While in early stages of infection the fungus may be confined to the vascular tissue of the crown, it is not strictly a vascular parasite; it may gain entrance to the crown through the young, infected basal shoots or by way of infected bases of hollow stems, which are ideal infection centers for the initiation of a crown rot. In the spring, badly decayed crowns send up a few weak, sickly shoots from uninvaded buds, but these soon wilt and die because there is not sufficient root system remaining to support them. A tug with the hand will uproot such plants.

The fungus is also found on the terminal portions of the inflorescence, especially during the latter part of the season. With prolonged moist weather the inflorescence and seed pods may become involved in a blackish decay.

GENERAL MORPHOLOGY AND TAXONOMY OF THE FUNGUS

Diplodina delphinii produces a heavy, septate mycelium that frequently branches and anastomoses, and grows well on a number of agar media. On potato-dextrose agar the fungus colony is very compact, with thick tufts of aerial hyphae. Pycnidia appear in 4 to 5 days and are fairly abundant on most of the media on which the fungus has been grown. The pycnidia are arranged irregularly over the surface of the medium, and as the culture ages and the aerial mycelium becomes appressed, they are evident in large numbers on the top of the colony. They are mostly oval to spherical but may be elongate, usually having one ostiole but sometimes two or more. On agar media the pycnidia are smaller than those formed on autoclaved delphinium leaves and stems and on other vegetable substrata. On autoclaved leaves and potato plugs, large, thick-walled, dark brown chlamydospores or chlamydosporelike cells are formed as the cultures age. One to several adjacent cells, either terminal or intercalary, become enlarged, thick-walled, and darker in color than the hyphal cells from which they originate (Fig. 1, H, I, J, K). Adjacent chlamydospores separate readily and thus would appear to function as individual resting cells of the fungus. They have also been observed on cornmeal agar cultures, after 5 days' growth. Here they arise in intercalary fashion, from highly colored mycelium rich in oil drops (Fig. 1, G). Similar chlamydosporelike cells have been observed in nature.

The pycnospores from pycnidia on naturally infected plants are considerably larger and differ further from those produced in culture in that they are mostly 1-septate, occasionally 0-, 2-, and rarely 3-septate (Fig. 2, E, F, G). The marked contrast between the large size and septate character of the pycnospores formed in nature and the small aseptate pycnospores (Fig. 2, H) produced on laboratory media seemed at first to indicate that more than one fungus was involved in the disease. However, a study of single-spore isolates demonstrated conclusively that these differences merely indicate the variability of the fungus when growing under different conditions. A number of 0-, 1-, and 2-septate spores were isolated singly from pycnidia on infected stems and grown on a wide variety of agar media, including potato-dextrose, cornmeal, malt, carrot, Lima-bean, and Sabouraud's maltose agar, and on autoclaved delphinium leaves and stems, potato and carrot plugs, bean, pea, and string-bean pods. During germination, the spores swell considerably and send out one or more germ tubes from each cell (Fig. 2, A, B, C, D). Pycnidia were formed on all of the mentioned substrata, but in all instances only one-celled pycnospores were found in the mature pycnidia. Furthermore, when a number of these same isolates were inoculated into healthy seedlings, the pycnidia formed on the lesions bore mostly 1-septate but also 0- and 2-septate spores. This variability is a phenomenon common to several members of the Sphaerioidaceae. The fungus *Stagonospora curtisii*, the cause of leaf scorch of narcissus, was

described under several specific names distributed among several genera, for the reason that the extreme variability of the fungus under both field and laboratory conditions was not understood until comparatively recently. Creager (2) found that the pycnospores produced by *S. curtisii* on nar-

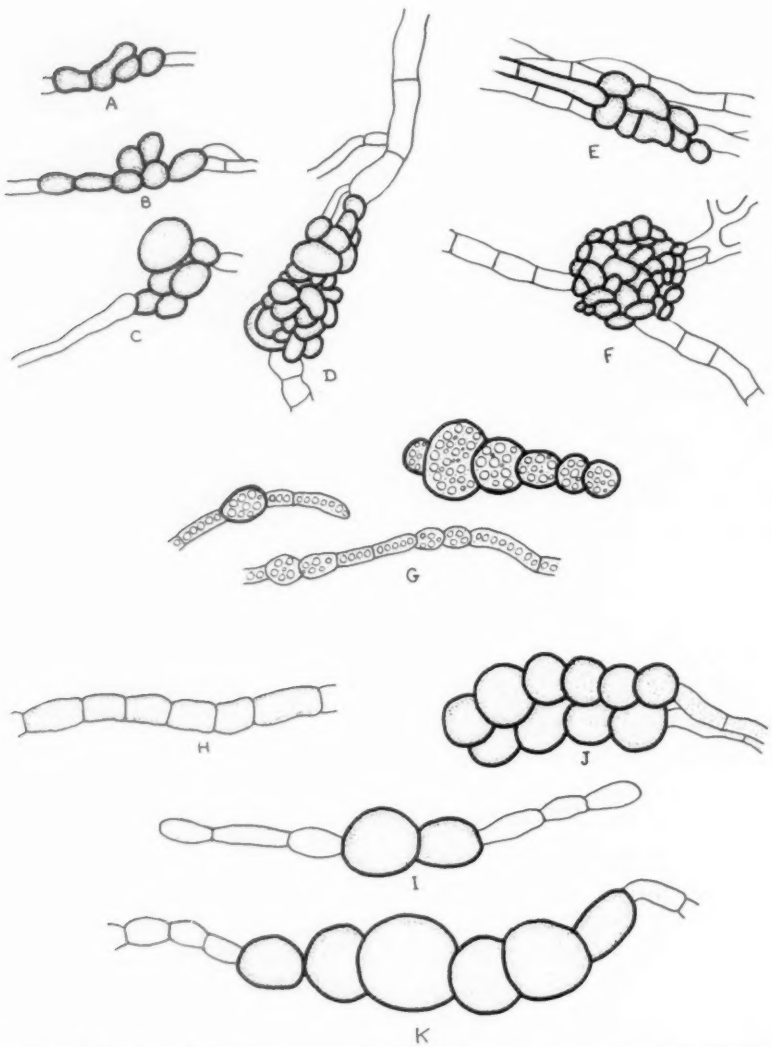

FIG. 1. *Diplodina delphinii.* A, B, C, E: Early stages of pycnidial development. D, F: Multicellular pseudoparenchymatous stages of pycnidial development. G: Chlamydospores arising from highly pigmented young hyphal cells containing large numbers of oil globules. H, I, J, K: Chains of chlamydospores.

cissus-bulb agar, autoclaved narcissus leaves, and in living leaves in the greenhouse were very small, nearly all 0-septate, with 1-septate ones occurring rarely. Spores taken from leaves in the field, however, were much larger and the majority were 3-septate, although 1-septate spores were pres-

ent, while 0-septate spores were relatively rare. Sprague (6) in describing the morphological characters of *Ascochyta boltshauseri* states that although the pycnospores were predominantly 1-septate, 15 to 20 per cent of the spores were 2- to 5-septate, mostly 2-septate. Despite this variation in the fungus, Sprague considered that since the most common condition of mature field material of this fungus is that of an *Ascochyta,* it should be assigned to that genus. The genera *Ascochyta* and *Diplodina* are very similar and both are characterized by the predominance of 1-septate spores.

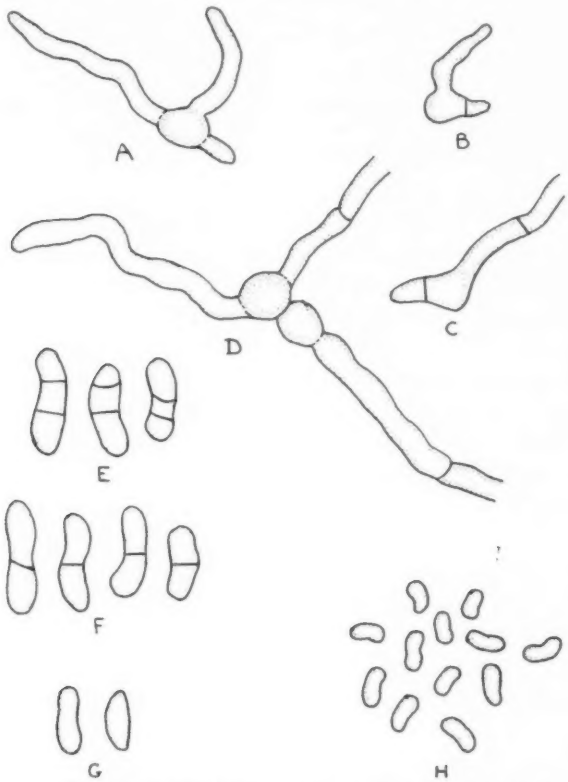

FIG. 2. Spores of *Diplodina delphinii.* A, B, C, D: Germinating spores. E, F, G: 2-, 1-, and 0-septate spores from pycnidia on the host. H: Spores from pycnidia on potato-dextrose agar.

Were the delphinium fungus to be placed taxonomically on the basis of its morphology under conditions of artificial culture alone, it would unquestionably be regarded as a species of the form genus *Phoma*, and in his preliminary report (4) the writer so designated it. However, since the fungus under conditions of natural infection produces pycnidia that typically and characteristically contain a predominance of 1-septate spores, it is now believed the fungus should be assigned to the genus *Diplodina*, family Sphaerioidaceae of the order Sphaeropsidales of the Fungi Imperfecti. Inasmuch as the fungus has not been previously described or re-

corded on delphinium, it is described as new and the following name is proposed:

Diplodina delphinii, sp. nov.[3]

Pycnidia in nigris aut brunneis lacerationibus irregularibus magnitudine formaque in caulibus, pedunculis, foliorum laminisque fictis; superficie item in moles rubrobrunneas in post maturitatem caulibus dispositis; pycnidia gregaria, saepe in concentrices moles disposita, epiderme tecta; globosa, pallido- vel opaquo-brunnea, ostiolis unibus vel tribus, 120–270 μ latis, plerumque 175 μ. Sporae multae, hyalinae, erectae aut vix curvatae, ambobus finibus obtuse rotundatis, 0–3-septatis, plerumque 1-septatis (80–90 per cent); 0-septatis sporis 6.6–14.8 × 3.3–4.0 μ plerumque 9.8 × 3.5 μ; 1-septatis sporis 9.9–19.8 × 3.3–4.1 μ, plerumque 14.7 × 3.7 μ; 2-septatis sporis 14.8–19.8 × 3.3–4.0 μ, plerumque 17.1 × 3.8 μ. Habitat: Caulibus, foliis, petiolis, corona, radicibusque hybridarum delphinii.

In culturis: pycnidia multa, spherica vel ovalia, opaque-brunnea vel nigra, ostiolata (1–4 ostiolis), 110–175 μ lata, plerumque 120 μ. Sporae multae, hyalinae, ovales vel ellipsoideae, biguttulatae, semper 1-cellula, 3.2–6.8 × 1.3–2.6 μ, plerumque 4.3 × 1.6 μ. Chlamydosporae crassae opaquo-brunneae, aliquando intercalares et moniliformes, 13.2–29.7 μ latae, plerumque 18 μ latae.

Pycnidia formed on black to brown lesions of irregular size and shape on stems, leaf petioles, peduncles, and leaf blades; also superficially in reddish-brown masses on old stems. Pycnidia gregarious, often in concentric masses, covered by the epidermis; globose, light to dark brown, with one to three ostioles, 120–270 μ in diameter, average 175 μ. Spores numerous, hyaline, straight or somewhat curved, bluntly rounded at both ends, 0- to 3-septate, mostly 1-septate (80–90 per cent); 0-septate spores 6.6–14.8 × 3.3–4.0 μ, average 9.8 × 3.5 μ; 1-septate spores 9.9–19.8 × 3.3–4.1 μ, average 14.7 × 3.7 μ; 2-septate spores 14.8–19.8 × 3.3–4.0 μ, average 17.1 × 3.8 μ. Habitat: Parasitic on stems, leaves, petioles, crown, and roots of delphinium hybrids.

On culture media: pycnidia numerous, spherical to oval, dark brown to black, ostiolate (1–4 ostioles), 110–175 μ in diameter, average 120 μ. Spores numerous, hyaline, oval to ellipsoid, biguttulate, always one-celled, 3.2–6.8 × 1.3–2.6 μ, average 4.3 × 1.6 μ. Chlamydospores thick-walled, dark brown, intercalary or not, sometimes in chains, 13.2 to 29.7 μ in diameter, average 18 μ.

Type locality: Ridgefield, Connecticut.

Type specimens: Specimens have been deposited in the Mycological Herbarium of the New York Botanical Garden, New York City.

The perfect stage of the fungus was not found. Various methods of overwintering were tried but in no case did the sexual or perfect stage of the fungus develop.

DEVELOPMENT OF THE PYCNIDIUM

The development of the pycnidium was studied in hanging drops, in Petri dish cultures, and from sectioned material. Most often the primordium arises directly from mycelial cells of a main hypha. Adjacent cells swell and become darker and thicker-walled than the other cells of the

[3] Latin diagnosis prepared by Dr. John D. Dwyer of Fordham University.

hypha (Fig. 1, A). They then begin to divide in several planes, eventually forming a compact mass of parenchymatous tissue (Fig. 1, B, C, D). Side branches from neighboring cells of the same hypha grow out and become involved in the young primordium. Frequently branches from neighboring hyphae take part in the development of the pycnidial wall (Fig. 1, F).

According to Kempton (3), pycnidia originate and develop by two main methods, namely meristogenous and symphogenous. The meristogenous method may be either simple or compound. In the simple type, the pycnidium develops from a single cell or a few adjacent cells of a single hypha; in the compound type, adjacent cells of two or more continuous hyphae divide, swell (Fig. 1, E), sometimes branch, and then anastomose freely to form a pseudoparenchymatous mass. In the symphogenous type, which is less common in the Sphaeropsidales, branching hyphae from main mycelial threads are directed towards a common point, loop back, and interweave to form a loose network which later becomes close. The hyphae of this ball anastomose into a pseudoparenchymatous mass from which the pycnidium develops. The pycnidia of *Diplodina delphinii* are most commonly of the simple meristogenous type and less frequently of the compound meristogenous type.

Five-day-old cultures on cornmeal and Lima-bean agar plates were fixed in Flemming's weaker solution. Sections stained with the triple stain showed that the cells of the hyphae are multinucleate. The tissues at the center of the young pycnidium soon begin to break down to form a lysogenous cavity. The cavity gradually enlarges and the tissue within it disappears. The uninucleate pycnospores are cut off from outgrowths of the thin-walled, almost colorless cells which line the sides and bottom of the pycnidium. When nearly mature, the pycnidium is tightly packed with a solid mass of spores and shortly afterward ostiole formation ensues, preceded by the proliferation of the apical mass of buffer tissue. The wall of the pycnidium consists of a layer of from two to four cells.

COLONY CHARACTERISTICS

Pycnidia developed on all of the media on which the fungus was grown, but different isolates varied in ability to produce them on different media. Some isolates can apparently lose the ability to produce pycnidia on any of the media. Apparently correlated with this loss is the lack of heavily pigmented mycelium so characteristic for most isolates of this fungus. Generally, after 3 weeks' growth, the aerial mycelium becomes appressed, exposing the pycnidia. The agar medium becomes darker by the diffusion of staling substances. The surface mycelium and the submerged hyphae become highly pigmented and the underside of the colony appears black. Some isolates produce a diffusible green to olive-amber pigment on certain media (potato-dextrose, carrot, and Sabouraud's maltose agar).

EFFECT OF TEMPERATURE ON GROWTH OF THE FUNGUS

Tests were made to determine the relation of temperature to the growth of the fungus in culture. In each case, blocks of agar of uniform size were used to transfer the fungus to plates of potato-dextrose agar. The plates were held at 27° C. for 24 hr., then left in the various temperature chambers for one day before initial readings were taken. Three plates each of two monospore isolates, 97A and 107E, were used in each chamber. Readings were taken every 24 hr. Since there was close agreement between the series of tests made, data for one set only are included here (Table 1). The

TABLE 1.—*Growth rates of two Diplodina isolates on potato-dextrose agar at different temperatures*

Temperature (°C.)	Isolate 97A				Isolate 107E			
	Increase in diameter				Increase in diameter			
	First day	Second day	Third day	Average daily increase	First day	Second day	Third day	Average daily increase
	mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
4	1.7	1.7	2.5	1.9	2.7	2.3	1.8	2.3
10	5.0	5.8	4.8	5.2	5.8	5.6	5.6	5.7
14	6.0	6.8	5.8	6.2	6.5	6.8	6.5	6.6
15	7.0	6.8	7.3	7.0	7.3	7.6	7.0	7.3
20	10.8	10.6	11.3	10.9	10.6	11.0	10.6	10.7
25	11.7	11.7	12.0	11.8	11.8	12.3	11.8	12.0
27	8.5	10.0	9.8	9.4	11.3	11.8	11.0	11.3
31.5	0.0	0.0	0.0	0.0	2.7	3.0	0.3	2.0
35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

fungus was able to grow within a range from 4° C. to 31.5° C. Since no chambers of a lower temperature were available it was not possible to determine the exact minimum; however, 4° C. is very near the minimum. The optimum appears to be 25° C. although growth is rapid at 20° C. Isolate 107E was normal and representative of the bulk of the isolates; 97A was atypical, having lost the ability to produce highly colored mycelium. The difference between the two isolates was consistent in these tests, in that 97A was not tolerant of temperatures above 27° C., and had a slower growth rate than 107E.

PATHOGENICITY

Diplodina delphinii has been repeatedly isolated from plants in various stages of crown and root-rot decay from New York, New Jersey, and Connecticut. The constant occurrence of the fungus on the stems and its frequent isolation from decaying crowns and roots early indicated that it was probably a crown-decay parasite of considerable importance (Fig. 3).

The fungus was also commonly isolated from black leaf spots indistinguishable in appearance from those said to be caused by the bacterium *Phytomonas delphinii*. Table 2 shows the results of a number of isolations

made at different times of the season from leaves with black-spot symptoms. The spots cultured varied in size from incipient to very large. Of a total of 375 spots cultured, *Diplodina delphinii* developed from 315, or 84 per cent. In addition to the *Diplodina* fungus, a number of bacteria were isolated, often from the same lesions. Rarely, however, was any one bacterial type frequent enough to indicate that it was the cause of the black leaf spot. Six of these bacterial isolates were sprayed on the leaves of potted delphinium plants, but none caused any leaf-spotting. These observations indicate that the fungus *D. delphinii* was probably closely associated with what is commonly known as black leaf spot of delphinium, and

FIG. 3. Colonies of *Diplodina delphinii* developing from diseased crown tissue of delphinium plant in an early stage of crown rot.

point to the need of further studies to define its relation to the leaf spot caused by *P. delphinii*.

The pathogenicity of the fungus has been determined experimentally in the greenhouse and field. In greenhouse tests, groups of healthy young seedlings sprayed with suspensions of pycnospores and subsequently kept under moisture chambers for 48 hr. showed symptoms of disease 5 days after inoculation. When young seedlings are sprayed with spores and mycelium of the fungus the long petioles of the crown leaves are severely attacked. The initial infection spots are very numerous and often coalesce to involve the whole petiole in a general brown to black rot. Check plants sprayed with water alone and kept under the same conditions as the inoculated plants showed no symptoms of disease. The maintenance of a very humid and moist atmosphere appeared to be necessary, but injury to the plants was not essential for infection.

Symptoms on plants inoculated in the greenhouse were small, black stem lesions which enlarged rapidly to involve areas 2 cm. or more in length, and severe infection and collapse of leaf petioles. In time, the fungus spread down the stem and into the crown. Pycnidia were not produced in the young necrotic areas, but developed later in abundance.

TABLE 2.—*Results of isolations from delphinium leaves with various types of black leaf-spot symptoms*

Source material and number of spots cultured	Number of *Diplodina* colonies	Number of miscellaneous bacteria	Number of miscellaneous fungi	Number of plates with no growth
Naturally infected leaves				
36	24	4	2	6
63	54	5[a]	0	8
14	14	4[a]	0	0
29	22	5	6	0
30	27	15[a]	0	0
40	40	24	0	0
35	27	4	3	1
34	23	31[a]	0	2
16	12	1	2	1
20	18	1	1	0
23	22	13[a]	0	0
35	32	29	0	0
Totals 375	315	136[a]	14	18
Inoculated leaves				
12	12	0	0	0
24	24	0	0	0
Inoculated petioles				
16	16	0	0	0
Totals 52	52	0	0	0

[a] Includes bacterial colonies which developed simultaneously with *Diplodina delphinii* from individual spots.

The infection on the leaves appeared as numerous small brown water-soaked lesions mostly on the undersides of the leaves. The typical black-spot lesions with which the fungus is frequently associated on diseased material in the field were never observed in these greenhouse tests. Bryan (1) encountered similar difficulties in greenhouse inoculations with *Phytomonas delphinii*. It may be that in the greenhouse the fungus is unable to cause typical black-spot lesions on the leaves.

Most of the plants inoculated in the greenhouse were placed outdoors in late fall and allowed to become dormant. In the spring, a number of the plants were dead and most of the rest sent up a few shoots which wilted several days after emerging. The fungus was easily reisolated from such shoots and from the decaying crown tissues.

The fungus was unable to cause root rot when a 1-in. layer of soil inoculum in which the *Diplodina* was growing replaced the top layer of soil in potted delphiniums. None of the plants in soil thus infested became diseased. This result agrees with the observations made of the disease in the

field, as the fungus is found most often associated with plants in various stages of crown rot which evidently began as decay of the stems, rather than as root rot.

In the field, four beds of 50 plants each were sprayed at various times during the season with water suspensions of the fungus pycnospores. A cheesecloth tent over the inoculated plants maintained conditions optimum for infection for 5 days. The fungus became established to some extent on the stems of a number of plants in all of the inoculated beds. Although the inoculations were not so successful as desired, the leaves and petioles of the plants in one bed developed numerous large, tar-spot lesions, mostly on the undersides of the leaves. Isolations made from these lesions yielded pure cultures of *Diplodina* (Table 2). Only a few plants died of crown rot and from these the fungus was recovered. It was not expected that the fungus would cause a severe crown rot of these plants the same season they were inoculated, because in natural infection the fungus requires a longer time to bring about a crown-rot disease. Under field conditions the crown rot usually was found on plants 2 or more years old.

Observational evidence indicated that the disease is favored by excessive moisture and is more active on plants weakened by excessive feeding. Since massive spikes are usually desired, growers often resort to supplementary irrigation and feeding of the plants with rich, quickly available commercial fertilizers. Under such conditions the disease is especially prevalent.

CONTROL

No experimental work has been carried on for the control of the disease caused by *Diplodina delphinii*, but the following measures may aid in its control.

Sanitation. Since the fungus grows and produces pycnidia readily on the dead and decaying parts of the plants, the importance of their removal is evident, especially from around the basal parts of the plants.

Spraying with a fungicide such as Bordeaux mixture in early spring, as the leaves and shoots emerge, may help to retard the disease. Supplementary spraying throughout the season may control black leaf spots and stem cankers and thus prevent the spread of the fungus to the crown. At the present time, delphinium growers as a rule spray only for the control of insect pests, and dust with sulfur for the control of mildew.

The plants should not be fed or watered excessively because lushness of growth favors the progress of the disease.

SUMMARY

A new disease of delphinium caused by *Diplodina delphinii* sp. nov. is described. In addition to crown rot, the fungus can cause a black leaf spot, petiole rot, and stem canker. Because of its wide prevalence in the delphinium plantings examined, the probable importance of this organism as

a source of crown rot losses is indicated. The pathogenicity of the fungus was demonstrated experimentally.

THE NEW YORK BOTANICAL GARDEN
AND
UNIVERSITY OF MINNESOTA

LITERATURE CITED

1. BRYAN, M. K. Bacterial leaf spot of Delphinium. Jour. Agr. Res. [U.S.] **28**: 261–270. 1924.
2. CREAGER, D. B. Leaf scorch of Narcissus. Phytopath. **23**: 770–786. 1933.
3. KEMPTON, F. E. Origin and development of the pycnidium. Bot. Gaz. **68**: 233–261. 1919.
4. LASKARIS, T. A new Phoma disease of perennial delphinium. (Abstr.) Phytopath. **30**: 19. 1940.
5. ————. Report of the delphinium crown rot investigation fellowship. Delphinium (Amer. Del. Soc.) **1940**: 102–108. 1941.
6. SPRAGUE, R. Ascochyta boltshauseri on beans in Oregon. Phytopath. **25**: 416–419. 1935.

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STABILITY OF FORMALDEHYDE SOLUTIONS USED IN BULB TREATMENTS[1]

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The hot-water-formalin treatment for the control of the bulb or stem nematode *Ditylenchus dipsaci* (Kühn) Filipjev is widely used in the major bulb-growing areas of the world (1, 2). The temperature of the bath is maintained at 43° C. (110° F.) (2). The dilution of the U.S.P. formaldehyde (37 per cent formaldehyde by weight) is 1 to 200 (1 pt. to 25 gal. water). The time of treatment of the bulbs varies from 1 hr. for iris to 4 hr. for narcissus (2). The main purpose in using formaldehyde in the treating bath is to prevent the spread of disease; however, Courtney (2) has reported that a better kill of nematodes is obtained with formaldehyde solution than by hot water alone. The purpose of the present investigation was to determine what change, if any, occurred in the formaldehyde concentration of solutions during heat treatment of bulbs, and the factors responsible.

STABILITY OF SOLUTIONS WITHOUT BULBS IN SMALL EXPERIMENTAL TANKS

Procedure. A small experimental tank having a capacity of 40 liters (10.57 gal.) of solution and 9.1 kg. (20 lb.) of bulbs was used. The tank was heated electrically and the fluctuation of the temperature was controlled by thermostat to ± 0.5° F. A series of propellers and baffles kept a uniform temperature in all portions of the tank. Samples of the solution were taken in tightly closed screw-cap bottles. The formaldehyde content of these was determined by the method of Ripper (5), since this method seemed to be less subject to interferences than other methods investigated. Formic acid does not interfere with this titration in amounts up to 0.5 per cent, which is more than would occur under conditions of this experiment. The recovery of known amounts of formaldehyde added to the bulb-treating solution averaged 100 per cent, the maximum deviation being 3 per cent.

The small tank described was filled with 40 liters of U.S.P. formaldehyde, diluted 1–200 with water. The tank was heated by electric coils to 43° C. and this temperature was maintained for 4 hr. The heating was continued until a total of eight consecutive 4-hr. periods was completed. The tank was allowed to cool overnight and was reheated the following morning. Samples of the solution were taken (a) before heating, (b) after each 4-hr. heating period, (c) following the cooling of the tank, and (d) at the end of the eight periods. Acidity in the samples was negligible,

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TABLE 1.—*Change in formaldehyde concentration in solutions during eight 4-hr. heating periods in the small experimental tank with no replacement solution added*

Period No.	Trial 1		Trial 2	
	Dilution (by analysis)	Percentage of original concentration	Dilution (by analysis)	Percentage of original concentration
Original	1–185		1–186	
After period 1	185	100	185	100
2	182	102	183	102
3	181	102	181	103
4	179	103	180	103
5	175	106	175	106
6	172	107	173	107
7	169	109	172	108
8	168	110	169	110

showing no loss of formaldehyde by oxidation to formic acid. The entire test was duplicated.

Results. The data (Table 1) indicate that the concentration of formaldehyde actually increased slightly during the heating of the tank. Both water and U.S.P. formaldehyde were lost during the two trials (Table 2), loss of formaldehyde being proportionately less than that of water.

TABLE 2.—*Comparative loss of water and formaldehyde after eight 4-hr. heating periods in the small experimental tank*

Trial	Material	Amount in grams		Percentage lost	Ratio: $\frac{\text{gm. } H_2O \text{ lost}}{\text{gm. HCHO lost}}$
		At start	At end		
1	Water	40,000	31,100	22.3	
	USP HCHO	217	192	11.5	356
2	Water	40,000	31,300	21.7	
	USP HCHO	216	192	11.9	363

STABILITY OF SOLUTIONS WITH A LOAD OF BULBS IN SMALL EXPERIMENTAL TANK

Procedure. The same tank and procedure were used except that a fresh 20-lb. change of narcissus bulbs was added at the beginning and removed at the end of each 4-hr. heating period. An analyzed solution of formaldehyde of an approximate 1–200 concentration was added when necessary to replace that solution adhering to the bulbs and lost by evaporation. This trial also was repeated. In effect, these runs were small-scale replicas of commercial treatments, except that the tank was heated electrically.

Results. The concentration of formaldehyde in each case (Table 5) was slightly higher at the end of the eight 4-hr. heating periods than it was at the start.

STABILITY OF SOLUTIONS IN A 150-GALLON TANK WITH A LOAD OF BULBS

Procedure. An electrically heated treating tank holding 150 gal. of solution was used in this part of the experiment. The details of construc-

TABLE 3.—*The effect of added bulbs on the change in formaldehyde concentration of solutions in the small experimental tank*

Period No.	Liters of replacement added		Dilution (by analysis)		Percentage of original concentration	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Original			1–224	1–205		
Replacement			231	204		
After period 1	1	2	224	204	100	101
2	1	2	223	204	100	101
3	2	1	218	202	103	102
4	1	3	210	201	107	102
5	1	2	206	199	109	103
6	1		205	199	110	103
7		4	215	196	105	105
8			220	198	102	104

tion are given elsewhere (3). Narcissus bulbs were treated for five 4-hr. runs and Croft lily bulbs were treated for several 1-hr. runs. The temperature in each case was 43° C.

Results. In this large electrically heated tank the concentration of formaldehyde remained about the same, showing no loss of formaldehyde on repeated use (Table 4).

TABLE 4.—*Stability of formaldehyde solution in large electrically heated tanks after treatment of narcissus and Croft lily bulbs*

Bulb lot and period No.	Dilution (by analysis)		Percentage of original concentration	
	Trial 1	Trial 2	Trial 1	Trial 2
Narcissus				
Original	1–207	1–203		
After period 1	209	203	99	100
2	211	205	98	99
3	216	207	96	98
4	216	203	96	100
5	211	201	98	101
Croft lily				
Original	1–200	1–197		
After 3 periods	201	205	100	96
6 periods	195	193	102	102[a]
9 periods	195		102	

[a] Five runs rather than six.

STABILITY OF SOLUTIONS UNDER CONDITIONS OF COMMERCIAL TREATMENTS

Procedure. In order to check the change in formaldehyde concentration under large-scale conditions, arrangements were made to obtain samples from the treating tanks of commercial growers. Commercial tanks of about 1,200-gal. capacity, of the type described by Courtney et al. (3), were heated by passing live steam directly into large cement tanks. Temperatures were observed by means of a recording thermograph and controlled

by a steam valve; proper distribution of heat was insured by use of propellers and series of baffles. Crated bulbs were loaded into the tanks by means of an overhead pulley system.

Samples were taken before each run and after any addition of either water or U.S.P. formaldehyde. Before analysis, it was necessary to filter the samples to remove dirt. Some color remained in the solutions, but this did not interfere with the titrations. The analysis was carried out as for the preceding samples.

TABLE 5.—*Change in formaldehyde concentration of solutions in commercial bulb-treating tanks*

Series and tank No.	Period No.	Water added (gallons)	U.S.P. HCHO added (pints)	Dilution (by analysis)	Percentage of original concentration
I, Tank 1	Original	1200	48	1–250	
2	Original	1250	48	284	
1	1			255	98
2	1	60	2	290	98
1	2	30		284	88
2	2	300	8	326	87
1	3	30	2	309	81
2	3			389	73
1	4	30	1	324	77
2	4	30		425	67
II, Tank 1	Original	1200	48	211	
2	Original	1250	48	224	
1	1			219	96
2	1	30	1	231	98
1	2			217	97
2	2			255	88
1	3	90	2	238	89
2	3		1	261	87
1	4	50		259	82
2	4			251	90
2	5	60	2	263	85
2	6	30	1	273	82
2	7			279	81

Results. In each case (Table 5) there was a decrease in concentration of formaldehyde. This could be accounted for partly but not entirely by the addition of replacement solution more dilute than 1–200.

DISCUSSION

The results of the experiments with electrically heated tanks all show little or no loss of formaldehyde on heating. In part one, where quantitative measurements were possible, the amount of U.S.P. formaldehyde lost by evaporation was small in comparison to the amount of water lost. This is in agreement with the theory that formaldehyde occurs in solution in the hydrated form. One molecule of water unites with formaldehyde to form methylene glycol, which has a low vapor pressure (4, 6). The decrease in concentration of formaldehyde in the large commercial tanks may be due in part to heating the tank with condensed steam. Thermal calculations indicate that the minimum amount of water condensed to raise the tem-

perature of a solution 10° F. would be 1 per cent of the volume. This is largely independent of the steam pressure, since steam at low pressure has a slightly higher heat content than at a higher pressure. This figure does not take into account the heat lost by evaporation, heat loss into the walls of the tank, or the water added by condensation in the steam line. Neither does it include the amount of steam added to hold the temperature at 43° C. These factors apparently cause the dilution recorded in the commercial tanks.

SUMMARY

The loss of formaldehyde from a solution in a small experimental electrically heated treating tank without bulbs was 11.5 per cent, the loss of water 22 per cent in eight 4-hr. heating periods. The concentration in the remaining solution increased 10 per cent.

The same tank when loaded with fresh narcissus bulbs maintained its original concentration through eight 4-hr. runs when the replacement solution contained the same amount of formaldehyde as the solution in the tank.

Using a larger electrically heated tank, the formaldehyde concentration remained the same through five 4-hr. heating periods with narcissus bulbs, or nine 1-hr. runs with Croft lily bulbs.

In commercial tanks heated by steam, the concentration of formaldehyde was from 81 to 67 per cent of the original concentration in from four to seven 4-hr. heating periods. This is believed to be mainly due to dilution of the solution with the condensed steam from the heating operations.

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LITERATURE CITED

1. CHITWOOD, B. G., and F. S. BLANTON. An evaluation of the results of treatments given Narcissus bulbs for the control of the nematode *Ditylenchus dipsaci* (Kühn) Filipjev. Jour. Wash. Acad. Sci. **31**: 296–308. 1941.
2. COURTNEY, WILBUR D. When and how to treat bulbs for nematode control. Proceedings of the 1948 Bulb Growers Short Course. Northwest Bulb Growers Association.
3. COURTNEY, W. D., E. P. BREAKEY, and LOYD L. STITT. Hot-water tanks for treating bulbs and other plant materials. State College of Washington, Pop. Bul. 184. 1947.
4. PIRET, E. L., and M. W. HALL. Distillation principle of formaldehyde solutions. Ind. and Eng. Chem. **40**: 661–673. 1948.
5. RIPPER, M. Eine allgemein anwendbare, massanalytische Bestimmung der Aldehyde. Monatsch. 21: 1079–1084. 1900. Cited in: Analyse und Constitutionsermittelung Organischer Verbindungen, page 633, Hans Meyer. J. Springer, Berlin, 1909.
6. WALKER, J. F. Formaldehyde. Reinhold Publishing Corp., New York. 1944.

ARCTIC AEROBIOLOGY. III. THE PRESENCE OF SPORES OF CEREAL PATHOGENS ON SLIDES EXPOSED FROM AIRPLANES IN 1947[1]

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Spores of many species of fungi are adapted for aerial dissemination and are readily set free into the air. When picked up by rising air currents they may be carried to considerable heights where strong winds may bear them many hundreds of miles. Spores of pathogenic fungi may thus be carried into new areas and fresh outbreaks of disease may follow. The relation between this air-borne dissemination and epiphytotics has been recently discussed by Stakman and Christensen (6).

In 1933 Lindbergh, working with Meier (3), exposed 26 slides in his flight across the North Atlantic, ten of them over Greenland, of which six were north of the Arctic Circle. Their preliminary report (2) noting the presence of fungus spores and hyphal fragments on the slides is the only record of such spores in the Arctic air.

Among the more important air-borne plant disease fungi are some that are found on our economic plants, particularly cereals, such as *Puccinia graminis* Pers. causing stem rust of wheat and other cereals and grasses; *P. triticina* Erikss. causing leaf rust of wheat; *Helminthosporium sativum* P., K., and B., and *Helminthosporium* spp. causing root rot and leaf spots of cereals and grasses; and *Tilletia* and *Ustilago* spp. causing smuts. This paper is a report on the fungus spores, especially of the organisms just mentioned, that were collected on vaselined slides exposed from aircraft for 5 min. at 4000–5000 ft. altitude, over various parts of the Arctic and Subarctic. Concurrently, Petri plates with nutrient agar were also exposed, but for only 2 min. Both the slides and plates were exposed by Prof. N. Polunin and a brief account of the trip has been made (5). The data on air speed, altitude, latitude, and longitude has been published for the nutrient plates (4). As the slides were exposed during the above-mentioned trip, it is unnecessary to repeat that information here.

MATERIALS AND METHODS

The apparatus used in making the exposures consisted of small cardboard boxes, each containing a vaselined slide, and a holder which was

[1] This investigation was undertaken by the Department of Botany, McGill University, Montreal, Canada, in cooperation with the Dominion Laboratory of Plant Pathology, Winnipeg, Canada. Contribution No. 996 from the Division of Botany and Plant Pathology Science Service, Department of Agriculture, Ottawa, Canada.

The assistance of the Royal Canadian Air Force in exposing the slides and plates is gratefully acknowledged: their fine cooperation in this and subsequent trips has made possible collections from areas not otherwise accessible.

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placed in the airstream. The box measured $1\frac{1}{2} \times 3\frac{5}{8} \times \frac{1}{4}$ in. and held a standard 1×3-in. vaselined glass slide, secured in position by a drop of paraffin at each end. The cardboard lid was in two sections, a short $\frac{3}{4}$–1-in. piece glued to the box and the remaining piece attached by a strip of adhesive tape serving as a hinge. A brass holder, just large enough to accommodate the box, was mounted at the end of a wooden rod $14 \times 1 \times 1$ in., with a safety catch fitted on the end. To expose the slide the lid was folded underneath the box, the opened box placed in the holder, safety catch fastened, and the holder positioned 10 in. from the fuselage and held or clamped for the 5-min. exposure. Following exposure the holder was brought into the plane, the box was removed and, with its lid closed, was carefully wrapped in waxed paper and secured by rubber bands. The slides were loaded and unloaded in a relatively spore-free room. For microscopic examination the slides were removed, a large 50×20-mm. cover glass was added, and by means of a mechanical stage the entire area (1100 sq. mm.) was examined for the presence of fungus spores. The findings were recorded under the following headings: stem rust, leaf rust, *Alternaria*, *Helminthosporium*, smut, and unidentified spores (table on figure 1). A magnification of 120 was used for the general survey of the slide, and a magnification of 600 for the study of individual spores. Slides 1–27 were exposed at 10-min. intervals, the remainder at 15-min. intervals. The air speed was about 115 m.p.h., and the altitude 4000–5000 ft. (4).

Exposures were made as follows: August 12, Nos. 1–11, between Canso Lake, Northwest Territories, and Cape Bathurst, N.T.; August 25, Nos. 13–27, Cambridge Bay, N.T., to Somerset Island, N.T.; August 26, Nos. 28–39, Cambridge Bay to Yellowknife, N.T.; September 5, Nos. 40–53, Yellowknife to Edmonton, Alberta. The most northerly exposures were during flights of August 12 and 26, the former extending from latitude 67°38′ N. to 70°02′ N., the latter from 69°32′ N. to 72°38′ N. In terms of the Arctic Circle, which is latitude 66°42′ N., twenty-seven exposures were made north of this parallel. In terms of the tree line, indicated on the map (Fig. 1) as a broken line, the three flights of August 12, 25, and 26 were all beyond the tree limit.

The slides were examined at the Dominion Laboratory of Plant Pathology, Winnipeg, Manitoba, and the results are given in figure 1. The location of each of the 52 exposures is indicated on the map by the slide number, and in the table at the lower right the numbers per slide of the various kinds of spores are recorded. In order to represent the distribution of the different spore types, each is given a symbol (see legend in figure 1) and the symbols are placed on the map opposite the slide number. Slides on which no objects were found are omitted from the table.

RESULTS

Except in the last flight the number of spores obtained on the vaselined slides was very low. Of the 38 slides exposed in the first three flights, 19

had no spores, 8 had but a single spore, 5 had only 2 spores, 3 had only 3 spores, and 3 slides had 4, 5, and 6 spores respectively. This indicates that the spore content of the air at the time of sampling was very low, and that

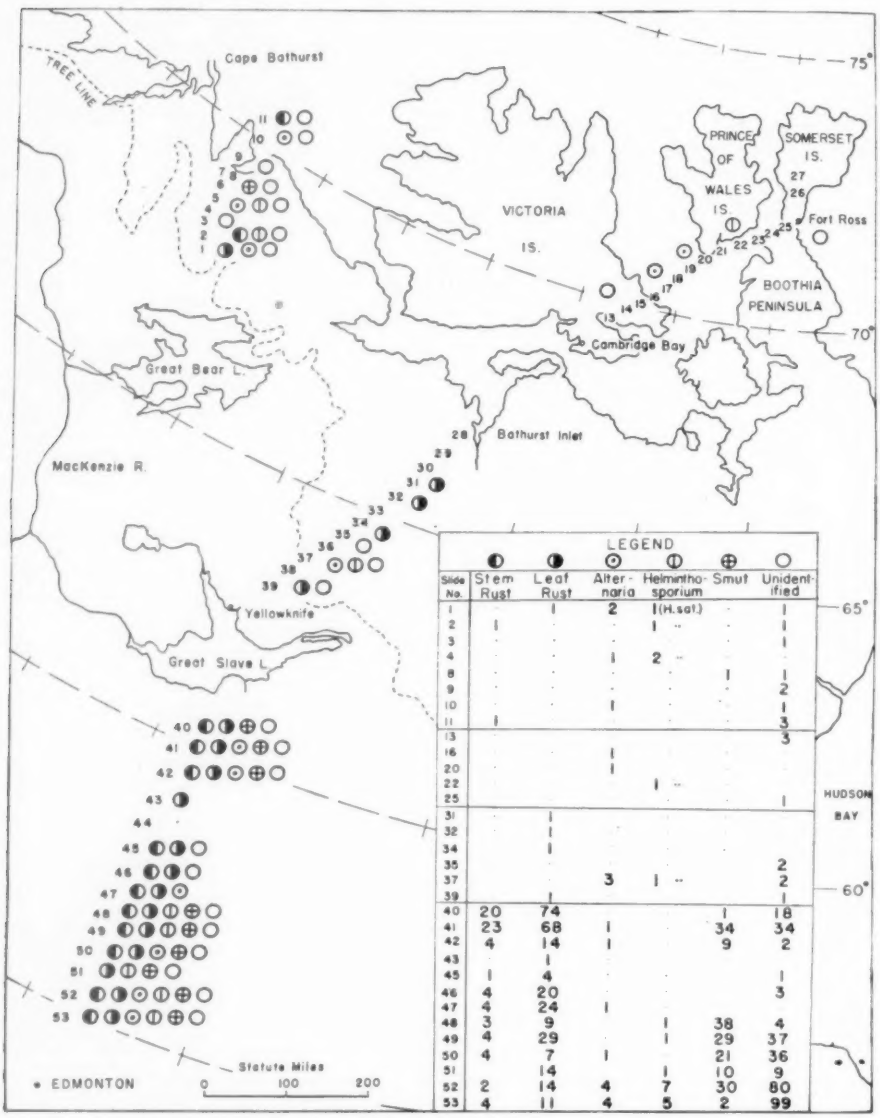
Cape Bathurst
TREE LINE
75°
PRINCE OF WALES IS.
SOMERSET IS.
VICTORIA IS.
Fort Ross
BOOTHIA PENINSULA
Cambridge Bay
70°
Great Bear L.
Bathurst Inlet
MacKenzie R.
Yellowknife
Great Slave L.
65°
HUDSON BAY
60°
Statute Miles
0 100 200
EDMONTON
LEGEND
Slide No. | Stem Rust | Leaf Rust | Alternaria | Helminthosporium | Smut | Unidentified
1 | | 1 | 2 | 1 (H.sat.) | | 1
2 | 1 | | | 1 | | 1
3 | | | | | | 1
4 | | | 1 | 2 | | |
8 | | | | | 1 | 1
9 | | | | | | 2
10 | | | 1 | | | 1
11 | 1 | | | | | 3
13 | | | | | | 3
16 | | | 1 | | | |
20 | | | 1 | | | |
22 | | | | 1 | | |
25 | | | | | | 1
31 | | 1 | | | | |
32 | | 1 | | | | |
34 | | 1 | | | | |
35 | | | | | | 2
37 | | | 3 | 1 | | 2
39 | | 1 | | | | 1
40 | 20 | 74 | | | 1 | 18
41 | 23 | 68 | 1 | | 34 | 34
42 | 4 | 14 | 1 | | 9 | 2
43 | | 1 | | | | |
45 | 1 | 4 | | | | 1
46 | 4 | 20 | | | | 3
47 | 4 | 24 | 1 | | | |
48 | 3 | 9 | | 1 | 38 | 4
49 | 4 | 29 | | 1 | 29 | 37
50 | 4 | 7 | 1 | | 21 | 36
51 | | 14 | | 1 | 10 | 9
52 | 2 | 14 | 4 | 7 | 30 | 80
53 | 4 | 11 | 4 | 5 | 2 | 99

FIG. 1. Map of region over which slides were exposed, showing locations at which the various kinds of spores were found.

the exposure was of too brief duration. This is well illustrated in the flight of August 26 when 10 of 15 slides were without spores and four had but a single spore, while only one, No. 13, had more than two spores.

It is of value to compare the total number of spores per slide with the

number of colonies obtained in the nutrient plates exposed at the same time, since the amount of air sampled by the plate exposed for 2 min. is approximately equal to the amount sampled by the slide in 5 min. (Table 1).

TABLE 1.—*Comparative results in 1947 of exposures of vaselined slides for 5 min., and standard nutrient plates for 2 min., in arctic and sub-arctic air. Slides 1–27 exposed at 10-min. intervals; 28–53 at 15-min. intervals*

Date and slide No.	Number of spores on slides	Fungus colonies on plate	Date and slide No.	Number of spores on slides	Fungus colonies on plate
Aug. 12			Aug. 26		
1	5	8	28	0	24
2	3	7	29	0	10
3	1	7	30	0	15
4	3	4	31	1	8
5	0	2	32	1	6
6	0	0	33	0	5
7	0	1	34	1	2
8	2	4	35	2	1
9	2	11	36	0	7
10	2	4	37	6	5
11	4	5	38	0	4
Aug. 25			39	2	2
13	3	12	Sept. 5		
14	0	14	40	113	19
15	0	9	41	160	33
16	1	8	42	30	6
17	0	7	43	1	3
18	0	5	44	0	7
19	0	6	45	6	11
20	1	8	46	27	18
21	0	3	47	29	11
22	1	6	48	55	32
23	0	6	49	100	67
24	0	8	50	69	79
25	1	102[a]	51	34	111
26	0	3	52	137	306
27	0	12	53	125	.....[b]

[a] Many secondary colonies from large sporulating Penicillium colony.
[b] No plate exposed.

It should be borne in mind that not all of the spores which land on the nutrient agar are capable of growth; this is true particularly of the rust spores, and possibly also of the smut spores as well. Small hyaline spores would probably be overlooked on the slide at the magnification used, whereas they might make definite colonies on the plates. Minute colored spores, such as those of *Penicillium* or *Aspergillus*, would probably meet the same fate. In general, the figures given in the table (Fig. 1) represent only those dark thick-walled spores which are visible under a magnification of 120.

From table 1 it is clear that there is broad agreement between the two types of collections, particularly in the flights on August 12 and September 5, but there is little or no correlation in the flights made on August 25 and 26. In the flight of August 12 the numbers are gradually less in the first half of the flight, the smallest numbers being in the vicinity of exposure No. 6 where the only nutrient plate free from fungus colonies was obtained,

but they increased gradually during the remaining portion. Slides 13–27 exposed on August 26, when the most northerly point was reached, had such a small number of spores that no conclusions can be drawn. That the air was, however, not devoid of fungus spores is evidenced by the presence of fungus colonies in all of the plates, ranging from 3 to 14 per plate and averaging 8. The large number of colonies recorded for plate 25 is due to secondary colonies from a sporulating Penicillium colony, and should be disregarded. In exposures 28–39, on August 27, this time traveling southward, the first 6 slides had a total of only 2 spores, 4 of them being free of spores, while the remainder had 11 spores on 4 slides, only 2 being without spores. On the other hand, the corresponding plates averaged 11.3 and 3.5 colonies respectively. It is difficult to account for the fact that there were more colonies on the first four plates than on any of the others, and the same appears to be true to some extent for each of the other flights.

The fourth flight, on September 5, yielded the highest number of spores (Fig. 1) as well as the largest number of colonies (Table 1). This is not unexpected as the last part of the flight was over northern Alberta and adjacent to, if not directly over, agricultural areas in the vicinity of Edmonton. Even in this flight the number of spores is not in a uniform series, the first slide, No. 40, having 113 spores, while the second, No. 41, had the largest number of spores caught on any slide. On the next slide, however, the numbers were appreciably less and the succeeding two slides were practically sterile. From 45 to 53 there is a general increase although none was as high as the second one. This peculiar situation is reflected also in the contemporary plates, the second plate likewise being much higher than its neighbors, with a sharp decrease to No. 43 gradually increasing through the remainder of the flight.

Uredospores of stem rust (*Puccinia graminis*) were uncommon in the first three flights, only two spores being found north of the Arctic Circle. Little significance can be attached to the presence of these single spores at such high latitudes, as they might easily be contaminants. On the other hand, uredospores were present in significant numbers on September 5, being found on all but three slides, yet unaccountably being especially numerous only on the first two slides. One teliospore of *Puccinia* sp. was found on slide 42.

Uredospores of leaf rust were somewhat more abundant, being found on four slides exposed north of the Arctic Circle, but they were not present in significant numbers. However, in the flight of September 5, uredospores were present in large and significant numbers on the majority of the slides (Fig. 1). In contrast to stem rust, uredospores of leaf rust were present in fairly high numbers on all slides except Nos. 43 and 44, the latter being negative. The evidence points to very high numbers of leaf-rust spores in the air in northern Alberta on this date.

Conidia of *Alternaria* are readily recognized by their large size, thick walls, and characteristic shape. Except in the two final exposures, they

could not be said to be abundant, but such spores as were present were widespread in their distribution. Spores of *Helminthosporium* are likewise easily identified, but they were only occasionally present except in the immediate vicinity of Edmonton. Of the 21 spores of *Helminthosporium*, six were identified as *H. sativum;* all were found in August, five of them north of the Arctic Circle. Smut spores (*Ustilago* and *Tilletia* spp.) were with one exception confined to the September flight. The sharp decrease in spore numbers in exposures 43–45 referred to earlier is strikingly evident here—large numbers on slide 41, slides 43–47 completely free; then a region of fairly high concentration is suddenly entered. A single spore of *Pleospora* was found on slide No. 1, and two spores of the Cladosporium type were found on slide No. 8. Unidentified fungus spores were present on 23 of the slides, the fewest occurring on the second flight; the fourth flight had unidentified spores on all but three slides, the last two slides having very high numbers. There is a sharp decrease in spore numbers between exposures 41 and 49, which is reflected both in the numbers of unidentified spores (Fig. 1) and in the number of colonies on the respective plates (Table 1).

Prevalence of Cereal Diseases in Western Canada in 1947

Stem rust of cereals and grasses. Stem rust of cereals and grasses (*Puccinia graminis* Pers.) was present in varying amounts on susceptible varieties throughout the agricultural area of Western Canada in 1947. Scattered infections began to appear in southeastern Manitoba during the first week in July. By mid-August stem-rust infection had become moderately severe on susceptible cereals throughout the agricultural areas of Manitoba and southeastern Saskatchewan. Elsewhere in the latter province it was present in trace amounts only. The first stem-rust infections in Alberta were observed at Lacombe on August 21. A few days later, local outbreaks of this rust occurred in some parts of southern Alberta; but elsewhere in that province stem-rust infection was scarce.

Leaf rust of wheat. Leaf rust (*Puccinia triticina* Erikss.) was present in 1947 on wheat throughout all the grain-growing areas of Western Canada. It appeared a week or more earlier than stem rust in most localities and was much heavier than stem rust in Manitoba and eastern Saskatchewan, where infections averaged upwards of 75 per cent in intensity with 100 per cent of the plants affected. However, in western Saskatchewan, infections of this rust were rather light. The quantity of leaf-rust inoculum present in Manitoba and eastern Saskatchewan was very much greater than that of stem rust, owing to the fact that most of the wheat acreage in that area was sown to wheat varieties that are susceptible to leaf rust and resistant to stem rust.

In Alberta, leaf rust was very much more prevalent than stem rust, and leaf-rust infection there became general and rather severe after the middle of August, particularly in the western half of the province. It would ap-

pear that the unusual abundance of leaf rust in Alberta was reflected in the number of spores caught on the slides exposed on September 5.

Crown rust of oats. Crown rust of oats (*Puccinia coronata* Corda) appeared in southeastern Manitoba in early July. A light infection of this rust developed throughout southern Manitoba and extended into eastern Saskatchewan. It was not observed in western Saskatchewan and Alberta. In the areas where this rust occurred it reached its maximum intensity at about the same time as did leaf rust of wheat.

Stripe rust. A widespread but light infection of stripe rust (*Puccinia glumarum* (Schm.) Erikss. and Henn.) occurred on winter wheat and susceptible grasses in southern Alberta.

Smuts. Bunt (*Tilletia* spp.) and loose smut (*Ustilago* spp.) were present throughout Western Canada. In most fields where smuts occurred, infections were present in trace amounts only. However, in most localities in Western Canada an appreciable number of fields had smut infections averaging one per cent or slightly higher.

Helminthosporium leaf and stem diseases. Leaf spots caused by *Helminthosporium* spp. were common on wheat and barley throughout Western Canada. In some fields in Alberta these diseases occurred in moderate amounts.

All the diseases discussed above occurred also in the central United States. In the northern parts of the States bordering the western Canadian Prairie Region, namely, Minnesota, North Dakota, and Montana, these diseases were present to about the same extent as in the southern parts of Manitoba, Saskatchewan, and Alberta.

Harvesting and Threshing Dates in Canada in 1947

Harvesting operations have a very marked effect on the number of spores of various plant-disease fungi that are dispersed into the air. At the time of harvest, particularly if the weather has been dry, many leaf and stem-rust spores are still present in the sori on leaves and stems of rusted cereal crops. When the reaper or combine moves through such a crop, clouds of spores are dislodged and either fall to the ground or are carried away by air currents. Similarly, during harvesting and threshing operations, the spores of other fungi causing leaf and stem diseases of cereals are dispersed; bunt balls are broken up; and opportunity for air dispersal of bunt spores is optimum.

Cutting and threshing of cereal crops in southeastern Manitoba usually begins during the first week in August, and from then on harvesting operations continue in parts of Western Canada until threshing is completed in northwestern Alberta in late September or early October.

On August 12, when the first group of slides was exposed over the Cape Bathurst–Canso Lake area, harvesting operations had been completed in the northern United States and were general throughout much of Manitoba but had not commenced in Saskatchewan and Alberta. On August 25 and 26,

when slides were exposed over the route from Somerset Island to Yellowknife, harvesting operations were still going on in Manitoba, had become general in most of Saskatchewan, and were under way in southern Alberta. On September 5, when the slides were exposed on the flight from Yellowknife to Edmonton, harvesting operations were nearly completed in Manitoba, and were general throughout northern Saskatchewan and in Alberta as far north as Edmonton.

DISCUSSION

Methodology is very important if reliable results are to be obtained (1). Stationary slides are commonly used in rust epidemiology studies, and the number of spores that accumulates in 24 hr. is regarded as an index of the number in the air. Such a method is useful where the primary purpose is to determine whether or not certain spores are in the air, and, if present, the approximate number, but it does not indicate accurately the number of spores in a given volume of air. When a slide is exposed from an airplane the results are even less satisfactory since the slide is now being carried forward rapidly through the air and is subject to additional variables, such as air speed and air flow around the slide. The method described here is subject to all of these criticisms, but it does provide some information on the presence or absence basis. Since the exposures were in an area where no previous work of this type had been done it seemed desirable to obtain as many data as possible despite the inadequacies of the method. The ideal method is one that combines quantitative as well as qualitative techniques. While aircraft appear to be well adapted for air sampling, there are several disadvantages: the high air speed makes it difficult to obtain a representative sample, while the addition of quantitative apparatus adds undesirable weight to the equipment. Despite these difficulties we have designed and are now testing an apparatus which appears to be satisfactory from both the qualitative and quantitative standpoints.

Extreme care must be exercised in interpreting data from slides on which the number of spores is low, because there is always the possibility of contamination during preparation, exposure, and examination. Of the 52 slides examined, 38 had fewer than five spores and 9 had but a single spore. This is offset to a large extent by the fact that 20 exposed slides bore no spores whatsoever, thus serving as controls. The indications are that there are very few spores in the Arctic air and that a 5-min. exposure is much too short. Lindbergh, working with Meier (2) in 1933, exposed for 40–60 min. over Greenland in the Arctic. On the only one of his slides for which data are available (exposed for 40 min. at 115 m.p.h. at 3000 ft.), the total number of objects was 193, representing 53 different types of objects, which are unspecified. Meier found spores of several genera, but *Puccinia, Alternaria, Helminthosporium, Ustilago,* and *Tilletia* were not represented.

Viability of air-borne spores of fungus pathogens is very important because of their disease-producing capacity. While fungus spores are fairly

resistant to the many factors which act upon them while they are in the air, it is reasonable to expect that there is a correlation between length of time in the air and decreased viability. The capture of large numbers of spores of pathogenic fungi on a slide is significant only if the percentage of viability is high. There is no method at present of determining whether or not the spores caught on the slides are viable or not. Some information may be obtained by exposing nutrient plates at the same time, but many fungi, such as the rusts and powdery mildews, are not cultivable on synthetic media, and others may require a special medium for germination and colony formation. There is need for more information on the resistance of single air-borne spores to such factors in the environment as temperature, moisture, and visible and ultraviolet light.

It seems likely that the air-borne population decreases northward; but it should be kept in mind that winds are capricious, and that mixing of a heavily spore-laden south wind that has been blowing for several days over agricultural areas, with a north wind from over the Arctic Ocean bearing few or no spores, would result in great variation in sampling. This is probably the explanation of the low numbers of spores encountered in the middle of the flights of August 12 and September 5 (Table 1). Even with improved techniques it is expected that considerable variation will be found. The incidence of cereal diseases in both United States and Canada is undoubtedly correlated with the numbers of spores in the local atmosphere, particularly at the time heavy sporulation is taking place, as in the uredostage of stem and leaf rust. That such spores are carried northward by the prevailing south winds is a well-known fact. Theoretically such spore clouds could be carried far into the Arctic Circle, but actually it is doubtful that they arrive there in large numbers, or, if they do, that they remain there. Cold fronts constantly pushing down from the Arctic Ocean bring in cool, relatively spore-free air, while precipitation removes the spores from the air. It is therefore problematical whether spores from epiphytotics in the southern agricultural areas will occur in representative numbers in the Arctic air.

SUMMARY

In four flights during August and September, 1947, in the Arctic and Subarctic, 52 vaselined slides were exposed for 5 min. from aircraft at altitudes of 4000–5000 ft. A special holder positioned the slides 10 in. from the fuselage.

Spores of *Puccinia graminis, P. triticina, Helminthosporium sativum, Helminthosporium* sp., *Alternaria,* and smut spores were found in addition to many unidentified spores.

In two flights beyond the Arctic circle, and in the third which was beyond the tree line, spore numbers were low and probably not significant, 19 of 38 slides being negative; on the remainder, the highest number was six.

In the fourth flight, from Yellowknife, Northwest Territories, to Edmon-

ton, spores of all the above types were found in significant numbers, only one slide being sterile.

The associated cereal diseases in Canada and the United States are reviewed.

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LITERATURE CITED

1. THE COMMITTEE ON APPARATUS IN AEROBIOLOGY. Techniques for appraising the airborne populations of microorganisms, pollen and insects. National Research Council. Phytopath. **31**: 201–225. 1941.
2. MEIER, F. C. Collecting microorganisms from the Arctic atmosphere. With field notes and material by Charles A. Lindbergh. Sci. Monthly **40**: 5–20. 1935.
3. ———. Microorganisms in the atmosphere of Arctic Regions. (Abstr.) Phytopath. **25**: 27. 1935.
4. PADY, S. M., C. D. KELLY, and N. POLUNIN. Arctic Aerobiology. II. Preliminary report on fungi and bacteria isolated from the air in 1947. Nature **162**: 379–381. 1948.
5. POLUNIN, N., S. M. PADY, and C. D. KELLY. Arctic Aerobiology. Nature **160**: 876–877. 1947.
6. STAKMAN, E. C., and C. M. CHRISTENSEN. Aerobiology in relation to plant disease. Bot. Rev. **12**: 205–253. 1946.

PRODUCTION OF PLANT VIRUS INHIBITORS BY FUNGI[1]

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In 1925, Duggar and Armstrong (3) showed that crude extract of pokeweed (*Phytolacca decandra* L.) markedly reduced the infectivity of tobacco mosaic virus. Since that time other substances, including growth products of various fungi, juice from insects, and trypsin, have likewise been found to inhibit infection with tobacco mosaic or other plant viruses. Study of materials that inhibit virus infection is of interest from at least two points of view. First, if the inhibitory effect is due to inactivation of the virus, then something may be learned of the mechanism of infection which would be of value in control of virus diseases. Or second, if the inhibitory effect results from alteration of host susceptibility, something may be learned of the conditions required for virus multiplication in a living cell. As a preliminary to such study, this paper deals with results of a search for fungus products that are capable of inhibiting infection with one or another plant virus, and with the results of a study of the activity and properties of two such products.

REVIEW OF LITERATURE

The first report that a plant extract can have an inactivating effect on a virus was that of Duggar and Armstrong (3) in 1925, who showed that tobacco mosaic virus infectivity was completely inhibited when treated with sap of pokeweed (*Phytolacca decandra* L.). Since then, a number of plant extracts, including sap of cucumber (11), tobacco (6), snapdragon (6), spinach (6, 10), and beet (6, 10), and growth products of *Aspergillus niger* (5, 8, 9) and *Aerobacter aerogenes* (8) have been found to inhibit infection with tobacco mosaic virus. Some, if not all, of these extracts also inhibited infectivity of other viruses, including that of potato ring spot (5, 9), cucumber mosaic (9, 10), tobacco ring spot (5, 9), potato veinbanding (9), and bean mosaic (5). The efficiency of the extracts in reducing infectivity depended upon the nature and concentration of the extract. In general, rather high concentrations were required to cause a measurable decrease in virus activity. Normal rabbit serum (2, 12), ovalbumin (2), milk (2, 5), and bovine serum (5) were shown to inhibit infection with tobacco mosaic, cucumber mosaic, and tobacco ring spot. Small amounts of trypsin completely inhibit infection with tobacco mosaic, cucumber mosaic, severe etch, aucuba mosaic, and tobacco ring spot (16). According to Stanley (16) the effect of trypsin is due to an action on the host plant, but according to

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Johnson (9) and Fulton (5) it results from direct action on the virus. Mustard oil, nicotinic acid, and atropin have been found to inhibit infection with tobacco mosaic virus (4). Black (1) has shown that an extract of clover leafhoppers inhibits infection with potato yellow dwarf, potato ring spot, turnip mosaic, tobacco necrosis, and tobacco ring spot viruses, only small quantities of the extract being required for complete inhibition.

MATERIALS AND METHODS

Fungus cultures. The organisms used in this study were selected on the basis of their availability and ability to grow on a sterilized medium made up to one liter with 40 gm. of cornmeal, 20 gm. of dextrose, and 4 gm. of Bacto beef. Stock cultures were grown on agar. About 200 ml. of the corn-dextrose-beef medium in a penicillin bottle was inoculated with a loopful of spores from the stock culture suspended in sterile water. The inoculated flasks were incubated at room temperature (20°–28° C.) for three weeks. At the end of this period, the contents of the flasks were shaken well, ground in a mortar, and pressed out through two layers of cheesecloth. The extract was then centrifuged at 3000 r.p.m. for 15 min. The supernatant liquid was used as a test solution. Filtrates were kept frozen at 5° F. when not in use and were adjusted to pH 7 by addition of buffer before being used.

Viruses. Tobacco mosaic (TM), southern bean mosaic (SBM), and tobacco necrosis (TN) viruses were used in the studies to be reported. They were selected because of the readiness with which they can be transmitted by mechanical means, because methods are available for testing their relative activities, and because they are among the most stable viruses known.

Virus stocks were maintained in infected plants grown in a greenhouse. Whenever a virus solution was required for testing, it was obtained by removing leaves from the infected plants and pressing the sap from them through two layers of cheesecloth. Southern bean mosaic virus was maintained in the Bountiful variety of garden bean (*Phaseolus vulgaris* L.), while tobacco mosaic and tobacco necrosis viruses were maintained in Turkish-type tobacco (*Nicotiana tabacum* L.).

Local lesion hosts. Southern bean mosaic virus produces necrotic lesions in plants of the Kentucky Wonder (15) and other varieties of garden bean (17). Similarly, tobacco mosaic virus produces necrotic local lesions in the Early Golden Cluster variety (13) and in leaves of *Nicotiana glutinosa* L. (7). Tobacco necrosis virus produces necrotic local lesions in leaves of Holmes' necrotic type tobacco (*N. digluta* × *N. tabacum*) and in leaves of the Kentucky Wonder and the Early Golden Cluster varieties of bean. These host plants were used for measuring virus activity in the present study. The plants were grown in composted soil in 4- or 6-in. pots in a greenhouse. Seed of bean were sown six to a pot. In about 8 to 10 days, the primary leaves were ready for inoculation. At this time, the plants were thinned, leaving three per pot. Necrotic type tobacco and *N. glutinosa*

seedlings were transplanted to pots and grown to a height of 8 to 10 in. All but five leaves were pinched off at the time of inoculation.

Method of testing inhibitory activity. Infectivities of the various virus preparations were determined by counts of the local lesions produced on appropriate host plants. Dilutions of the viruses were made in 0.1 M potassium phosphate buffer at pH 7. Inoculum was taken up on a small piece of white cheesecloth which was then rubbed over the test leaves. In most instances, one half of each leaf was inoculated with the treated solution and the opposite half with a standard control solution at the corresponding dilution, the test solution being placed on the left half-leaf in one set of plants and on the right half in an equivalent set of plants. The local lesions were counted on the fourth to sixth day after inoculation. In other instances the test and control solutions were compared on alternate leaves of the same host plant. Lesions that appeared were counted on the fourth to sixth day after inoculation. The difference in numbers of lesions produced by the test and control solutions was divided by the number produced by the control solution; the quotient, when multiplied by 100, was taken as the percentage reduction in infectivity.

EXPERIMENTAL WORK

About 50 species of fungi have been tested for ability to produce growth products capable of inhibiting infection by Southern bean mosaic, tobacco mosaic, or tobacco necrosis viruses. The organisms were allowed to grow in the corn-dextrose-beef medium for 3 weeks, after which a filtrate was obtained from them as previously described. The filtrate was mixed with an appropriate concentration of virus in the proportion of one part of filtrate to one of virus and eight of buffer. For most of the filtrates, the virus activity was tested immediately after mixing, after 1 hr., and after 24 hr., by inoculation of 12 leaves of suitable host plants. Alternate leaves or half-leaves of the same host plants were inoculated with a control solution. In all cases the percentage reduction after 0- and 1-hr. periods of contact was not significantly different from that after 24 hr.

Filtrates from 10 species of fungi caused inhibition of infection of 80 per cent or more with one or another of the viruses tested. Table 1 summarizes the percentage reduction obtained with these filtrates after 24-hr. contact with the virus.

The following species produced filtrates that caused less than 20 per cent reduction in infection with any virus: *Collybia velutipes, Helminthosporium victoriae, Lenzites saepiaria, Penicillium expansum, Sclerotinia minor, S. bataticola, Stromatinia smilacinae,* and *Trichoderma sp.* Since the method used to measure virus activity involves an error at times as great as 20 per cent it cannot be concluded that any of these species produced substances that are capable of inhibiting virus infection.

Filtrates of the following species were found to inhibit infection to the extent of 20 to 80 per cent with at least one of the viruses: *Alternaria caro-*

tae, Aspergillus quercinus, A. rugulosus, Botrytis sp., Chaetomium convolutum, Choanephora cucurbitarum, Colletotrichum cereale, C. lagenarium, C. lindemuthianum, Crucibulum vulgare, Daedalea confragosa, Daldinia concentrica, Diplodia zeae, Fusarium avenaceum, Gloeosporium bolleyi, Glomerella cingulata, Helminthosporium avenae, Lambertella viburni, Lenzites trabea, Monascus purpureus, Monilinia fructicola, Pleurage curvicolla, Polyporus pubescens, P. versicolor, Rhizoctonia solani, Sepedonium chrysospermum, Sordaria sp., Spondylocladium sp., Ustilago striiformis, Xylaria mali.

TABLE 1.—*Effectiveness of filtrates from various fungi and bacteria in inhibiting infection with Southern bean mosaic (SBM), tobacco mosaic (TM), and tobacco necrosis (TN) viruses*

Filtrate		Number of lesions (on 12 leaves)		Percentage reduction
		Virus plus filtrate	Virus alone	
Actinomyces sp.	(SBM)	65	597	89
Aspergillus niger	(SBM)	24	256	91
Botryotinia convoluta	(SBM)	169	499	66
B. convoluta	(TM)	67	1044	94
B. convoluta	(TN)	232	3165	93
Guignardia bidwellii	(SBM)	1	675	100
G. bidwellii	(TM)	4396	13739	68
G. bidwellii	(TN)	9214	14615	37
Lambertella pruni	(SBM)	154	6247	98
L. pruni	(TM)	1814	9880	82
L. pruni	(TN)	2468	9320	74
Memnoniella echinata	(SBM)	9	81	89
Neurospora sitophila	(SBM)	5	704	99
Pestalozzia sp.	(SBM)	329	477	31
Polyporus arcularius	(SBM)	13	107	88
Trichophyton mentagrophytes	(SBM)	626	4758	87
T. mentagrophytes	(TM)	386	3500	89
Trichothecium roseum	(TM)	147	6550	98
T. roseum	(SBM)	19	2880	99
T. roseum	(TN)	1830	6100	70

It is apparent from table 1 that the filtrates found effective for inhibition of Southern bean mosaic virus infection were not necessarily equally effective for inhibition of tobacco mosaic and tobacco necrosis virus infection. This was also true of the species whose filtrates caused between 20 and 80 per cent inhibition. There was a correlation between ability to inhibit infection with one virus and ability to inhibit another, but the correlation was not of a very high order. The question may be raised as to whether the variation between filtrates in ability to inhibit infection results from production by some fungi of growth products that are more highly effective on a weight basis than those produced by other fungi, or whether there is variation in the concentration of inhibitory substances produced. A quantitative study would be required to answer this question. In the meantime, it would seem advantageous in further research with plant virus

inhibitors to use those fungus cultures that are most highly effective in reducing virus activity. Only a few of the filtrates, notably those of *Trichothecium roseum* and *Neurospora sitophila*, caused visible burning of the leaves when applied in undiluted form.

Relationship Between Concentration of Filtrates and Inhibitory Activity

Because of the intensity of their effect, *Trichothecium roseum* and *Neurospora sitophila* were chosen for further study of their inhibitory activity. First of all, it was of interest not only to know the dilution end-points of the inhibitory substances present in the filtrate but also the relationship between filtrate concentration and inhibitory activity. Distilled water was used to prepare dilutions, differing by one-half log unit, of the filtrates and also of the sap of plants infected with SBM virus. Virus filtrate mixtures were then made by adding 1 ml. of molar phosphate buffer and 1 ml. of a given dilution of the filtrate to 8 ml. of the diluted virus. The infectivities of such mixtures on Kentucky Wonder bean plants were compared with those of corresponding control preparations.

Filtrates of *Trichothecium roseum* and *Neurospora sitophila* were effective in inhibiting SBM virus infection even when diluted 1000 times (Table 2). However, the effectiveness of this or other dilutions of the fil-

TABLE 2.—*Relationship of concentration of filtrate from Trichothecium roseum and Neurospora sitophila to reduction in infections produced on 12 leaves of Kentucky Wonder bean plants by various dilutions of Southern bean mosaic virus*

Log virus concentration	Fungus filtrate[a]	Log filtrate concentration				
		0.0	−0.5	−1.0	−1.5	−2.0
		(Percentage reduction in infectivity)				
−2.0	TR	...	100	98	77	69
	NS	93	97	88	67	51
−1.0	TR	...	98	94	70	29
	NS	96	87	66	36	0
0.0	TR	...	93	78	56	27
	NS	88	59	9	9	9

[a] Symbols TR and NS refer to *Trichothecium roseum* and *Neurospora sitophila*, respectively.

trates depended upon the virus concentration. When the final solution contained about 0.03 mg./ml. of virus (14), the filtrate at a dilution of 1/10 was capable of preventing 88 per cent of the infections. On the other hand, when the final virus concentration was about 0.0003 mg./ml., a 1/1000 dilution of the filtrates reduced infection by more than 50 per cent. The dilution end-point of the activity in the filtrates was therefore not established precisely.

When the percentage reduction in infectivity for a given concentration of virus is plotted as a function of the logarithm of filtrate concentration, the points lie along a curved line. When the logarithm of percentage re-

duction is similarly plotted a curved line again results. Apparently the relationship between filtrate concentration and inhibitory effect is a complicated one which only further study can resolve.

The filtrates of *Neurospora sitophila* and *Trichothecium roseum* caused no visible injury to the test plants except when applied in their most concentrated form. When used undiluted, they caused visible burning, indicating that they were toxic to the cells of the host at this concentration.

Relationship of Time of Contact between Virus and Inhibitory Agent to Reduction in Infectivity

In order to determine whether the inhibitory effect of the filtrate involved a time reaction, an experiment was made wherein a test solution consisting of a mixture of virus and filtrate from a culture of *Trichothecium roseum* and a control solution consisting of virus and sterile medium were inoculated at various intervals onto half-leaves of Kentucky Wonder bean plants. The percentage reduction in infectivity computed from the lesion counts is in table 3. The data show that inhibition took place immediately

TABLE 3.—*Relationship of time of contact of Southern bean mosaic virus with filtrate from Trichothecium roseum to reduction in numbers of lesions produced on 18 half-leaves of Kentucky Wonder Wax bean plants*

Time of contact (hours)	Total number of lesions (on 18 half-leaves)		Reduction (per cent)
	Test solution	Control	
0.0	5	90	94.5
1.0	14	179	92.2
2.0	26	240	89.2

on contact between virus and filtrate. There was no change in degree of inhibition during the 2-hr. period the virus was allowed to remain in contact with the filtrate. If the inhibitory effect involved a time reaction, the time required was too short to be detected.

Reactivation of treated virus. An experiment was made to determine whether the reaction leading to loss of infectivity could be reversed. A test solution was prepared by adding 1 ml. of SBM virus to 8 ml. of filtrate of *Trichothecium roseum* and 1 ml. of molar pH 7 phosphate buffer. A control solution was prepared by adding 1 ml. of virus to 1 ml. of sterile medium and 8 ml. of buffer. After intervals of 0.0, 1.0, and 2.0 hr., serial dilutions of the resultant test and control solutions were made by addition of 0.1 molar pH 7 phosphate buffer. The test and control solutions at equivalent dilutions were compared by inoculation of opposite half-leaves of Kentucky Wonder Wax bean plants. The results (Table 4) were essentially the same whether the solutions were diluted immediately or allowed to stand for 1 or 2 hr. before dilution. The fact that the dilution end-points for the test and control solutions were the same shows that the loss of virus

TABLE 4.—*Comparison of the infectivities of various dilutions of Southern bean mosaic virus with infectivities of equivalent dilutions of a mixture of virus and filtrate from a culture of Trichothecium roseum*

Hours of contact before inoculation	Local lesions on 24 half-leaves of Kentucky Wonder Wax bean plants									
	10^{-1}		10^{-2}		10^{-3}		10^{-4}		10^{-5}	
	V[a]	M[a]	V	M	V	M	V	M	V	M
0	435	10	34	21	2	2	1	0	0	0
1	256	6	19	26	2	4	1	1	0	0
2	403	9	31	31	2	5	0	2	0	0

[a] The symbols V and M refer to virus and a mixture of virus and filtrate, respectively.

activity resulting from the addition of *Trichothecium roseum* filtrate is reversible by simple dilution. Thus it can be concluded either that the filtrate enters into a reversible combination with the virus, or that it inhibits infection by altering susceptibility of the host. The data do not permit a choice between these conflicting explanations.

Effect of Filtrate of Tricothecium roseum Applied before Inoculation with Virus

An experiment was made in which half-leaves of Kentucky Wonder bean plants were first rubbed with a 1:10 dilution of filtrate from a culture of *Trichothecium roseum* and then at various intervals with SBM virus. As a control the opposite half-leaves were first rubbed with water and then with virus. A total of 48 lesions were produced on 12 half-leaves inoculated with the test solution, when the interval between application of filtrate and inoculation with virus was 10 min., as compared with a total of 265 lesions on 12 leaves inoculated with the control solution. This represents a reduction of 81 per cent in virus infectivity due to the inhibitory agent. At 20- to 30-min. intervals, the reduction obtained was 80 and 57 per cent, respectively. Thus, the application of filtrate from *Trichothecium roseum* to leaves prior to inoculation with SBM virus is effective in reducing the number of infections when the interval between application and inoculation is as great as 30 min. It appears, therefore, that prolonged contact of the virus with the agent is not necessary to inhibit infection. The results are similar to those obtained by Stanley (16), who found that trypsin sprayed onto leaves of *Phaseolus vulgaris* and *Nicotiana glutinosa* plants 6 days before inoculation with TM virus inhibited infection; and to those of Johnson (9), who found that the growth products of *Aerobacter aerogenes* applied to host plant leaves prevented infection for as long as a week.

Effect of Filtrate of Trichothecium roseum Applied after Inoculation with Virus

Leaves of Kentucky Wonder bean plants were inoculated with SBM virus. At intervals of 10, 20, and 30 min. after inoculation, one half of

each leaf was rubbed with a 1:10 dilution of filtrate from a culture of *Trichothecium roseum* and the other half-leaf was rubbed with water to serve as control. When the interval between inoculation and application of the filtrate was 10 min. the reduction in infectivity of SBM virus was 84 per cent, a total of 1470 lesions being produced on the 12 control half-leaves as compared with 226 on the 12 test half-leaves. At 20- and 30-min. intervals the reduction was 79 and 80 per cent respectively. Thus, the inhibitory agent in the filtrate of *T. roseum* was effective in reducing the number of infections when applied as long as 30 min. after inoculation with the virus. Apparently it was just as effective when applied 30 min. after inoculation as when applied 10 min. after. The action of the inhibitory agent present in the filtrate of *T. roseum* is similar in this respect to that of trypsin, which was found by Stanley (16) to be effective in inhibiting infection with tobacco mosaic virus when applied to leaves as long as 30 min. after inoculation. It is likewise similar to the action of growth products of *Aerobacter aerogenes* found by Johnson (9) to be even more effective than trypsin in reducing infection when applied after inoculation.

Properties of the Inhibitory Agents of Trichothecium roseum and Neurospora sitophila

Tests were made to determine the effect of temperature, lyophilyzation, and dialysis on the ability of filtrates of *Trichothecium roseum* and *Neurospora sitophila* to inhibit infection with SBM virus on Kentucky Wonder bean plants. In the heating experiment, undiluted filtrate of *T. roseum* was placed in thin-walled glass test tubes which were immersed in boiling water for 10 min., and a second sample of the undiluted filtrate was autoclaved at 10 lb. pressure for 10 min. The filtrates were allowed to cool and their inhibitory effect was then tested.

In another experiment, the filtrates of *Trichothecium roseum* and *Neurospora sitophila* were frozen and stored at −12° C. for 6 months. At the end of the period, the frozen material was thawed and its inhibitory activity tested.

In the lyophilyzation experiment, 20 ml. of filtrate of *Trichothecium roseum* was dried under vacuum at low temperature. An amorphous white powder was obtained. This material was resuspended in 10 ml. water and its inhibitory effect tested.

In the dialysis experiment, a 9-in. cellophane sausage casing containing a quantity of *Trichothecium roseum* filtrate was immersed in a flask of tap water and stored at 4° C. At the end of 24 hr. the water was changed. Another sausage casing containing only cornmeal-dextrose-Bacto beef broth was treated in the same manner. At the end of 24 hr. and again at the end of 48 hr. the liquids remaining inside the casing were tested for inhibitory activity. At similar intervals, filtrate that was not dialyzed but kept at 4° C. was also tested for inhibitory activity.

The data from these experiments, which are summarized in table 5, show that the inhibitory activity of the filtrate of *Neurospora sitophila* was not lost by boiling or by freezing and that the activity of the filtrate of *Trichothecium roseum* was not lost by boiling or autoclaving for 10 min., by freezing at – 12° C. for 6 months, or by lyophilyzation. They also show that the inhibitory activity was either nondialyzable or only slowly dialyzable under the conditions tested. In this latter respect the filtrate of *T. roseum* resembles that of *Aerobacter aerogenes* which possesses strong inhibitory activity (9). The variation in lesion counts produced by the controls is typical and is to be expected in experiments carried out at different times with different virus samples and various sets of plants.

TABLE 5.—*Effect of various treatments on the ability of filtrate from cultures of Trichothecium roseum and Neurospora sitophila to inhibit infection with Southern bean mosaic virus on Kentucky Wonder Wax bean leaves*

Filtrate from	Treatment	Number of lesions on 12, 16, or 18 half-leaves		Reduction (per cent)
		Virus plus filtrate	Virus alone	
T. roseum	Boiled	1379	7078	80.5
	Not heated	829	5390	84.6
N. sitophila	Boiled	4110	7055	41.7
	Not heated	4642	7255	36.0
T. roseum	Autoclaved	10	1298	99.7
	Not heated	15	2818	99.4
T. roseum	Frozen	6	474	98.7
	Not frozen	15	2818	99.7
N. sitophila	Frozen	107	830	87.1
	Not frozen	128	1029	87.6
T. roseum	Lyophilyzed	72	2726	97.3
	Nonlyophilyzed	387	4137	90.6
T. roseum	Dialyzed 24 hr.	203	2119	90.8
	Not dialyzed	8	2690	99.7
	Dialyzed 48 hr.	329	2990	89.1
	Not dialyzed	8	3061	99.7

DISCUSSION

A surprisingly large number of fungi produce growth products that are capable of inhibiting, to a greater or lesser extent, infection with Southern bean mosaic and tobacco mosaic viruses. About 75 per cent of the fungi tested actually were shown to produce such substances. Fungi vary tremendously in the potency, or concentration, of inhibitory materials in their growth products. The filtrates from only about 20 per cent of the cultures tested were found to decrease infection as much as 80 per cent.

The action of the inhibitory agents from *Trichothecium roseum* and *Neurospora sitophila* is similar in many respects to that of insect juices (1), trypsin (16), blood serum (9), and growth products of *Aspergillus niger*

and *Aerobacter aerogenes* (8, 9). In the case of all these materials, the reaction leading to loss of infectivity takes place immediately on mixing with virus. Moreover, in each case, the activity of a virus preparation rendered noninfectious by treatment with the inhibitor can be regained by simple dilution. It seems clear that the inhibitor either enters into a reversible reaction with the virus, or acts upon the host plant to alter its susceptibility. Although not conclusive, the available evidence favors the latter interpretation.

The fact that the activity of the inhibitory agent present in the filtrate of *Trichothecium roseum* is not destroyed by heat indicates that the agent is not a protein. The fact that the activity was retained by a cellophane membrane shows that the agent is of high molecular weight.

SUMMARY

Filtrates of 49 species of fungi growing on cornmeal-beef-dextrose broth were tested for presence of materials that would inhibit infection with Southern bean mosaic, tobacco mosaic, or tobacco necrosis viruses. It was found that 84 per cent of the fungi tested produce such inhibitory agents. However, less than 25 per cent of the fungus cultures were capable of reducing infectivity as much as 80 per cent. The fungi differed either in the kind of agent they produce or in its concentration.

Growth products of two fungi, *Trichothecium roseum* and *Neurospora sitophila*, both of which caused a 90 per cent or greater reduction in infectivity, were studied in some detail. They both caused immediate loss of infectivity when mixed with a virus solution. Both have been kept frozen for 6 months without loss of activity. The filtrate from *T. roseum* was effective in inhibiting infection when applied to host plant leaves before or up to 30 min. after inoculation with virus.

The infectivity of a noninfectious mixture of virus and filtrate was restored by simple dilution, suggesting either that the inhibitory agent enters into a reversible combination with the virus or that it alters host susceptibility.

The agent present in the filtrate of *Trichothecium roseum* was found to be thermostabile, nondialyzable, and lyophilyzable. It is therefore not of protein nature but is of large molecular size.

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LITERATURE CITED

1. BLACK, L. M. Inhibition of virus activity by insect juices. Phytopath. **29**: 321–327. 1939.
2. CHESTER, K. S. Specific quantitative neutralization of the viruses of tobacco mosaic, tobacco ring spot, and cucumber mosaic by immune sera. Phytopath. **24**: 1180–1202. 1934.
3. DUGGAR, B. M., and J. K. ARMSTRONG. The effect of treating the virus of tobacco mosaic with the juices of various plants. Ann. Missouri Bot. Garden **12**: 359–366. 1925.

4. FUKUSHI, T. Effects of certain alkaloids, glucosides and other substances upon the infectivity of the mosaic tobacco juice. Trans. Sapporo Nat. Hist. Soc. **11**: 59–69. 1930.
5. FULTON, R. W. The sensitivity of plant viruses to certain inactivators. Phytopath. **33**: 674–682. 1943.
6. GRANT, T. J. The host range and behavior of the ordinary tobacco mosaic virus. Phytopath. **24**: 311–336. 1934.
7. HOLMES, F. O. Local lesions in tobacco mosaic. Bot. Gaz. **87**: 39–55. 1929.
8. JOHNSON, J. Plant virus inhibitors produced by microorganisms. Science **88**: 552–553. 1938.
9. ———. Chemical inactivation and the reactivation of a plant virus. Phytopath. **31**: 679–701. 1941.
10. KUNTZ, J. E., and J. C. WALKER. Virus inhibition by extracts of spinach. Phytopath. **37**: 561–579. 1947.
11. MCKINNEY, H. H. Factors affecting certain properties of a mosaic virus. Jour. Agr. Res. [U.S.] **35**: 1–12. 1927.
12. MULVANIA, M. Studies on the nature of the virus of tobacco mosaic. Phytopath. **16**: 853–871. 1926.
13. PRICE, W. C. Local lesions on bean leaves inoculated with tobacco mosaic virus. Amer. Jour. Bot. **17**: 694–702. 1930.
14. ———. Purification and crystallization of Southern bean mosaic virus. Amer. Jour. Bot. **33**: 45–54. 1946.
15. ———, and B. R. HOLT. Kentucky Wonder bean plants as hosts for measuring Southern bean mosaic virus activity. Phytopath. **38**: 213–217. 1948.
16. STANLEY, W. M. Chemical studies on the virus of tobacco mosaic. 1. Some effects of trypsin. Phytopath. **24**: 1055–1085. 1934.
17. ZAUMEYER, W. J., and L. L. HARTER. Two new viruses of beans. Jour. Agr. Res. [U.S.] **67**: 305–328. 1943.

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THE UNSEASONED WOOD DISC METHOD FOR EVALUATING SAP-STAIN CONTROL CHEMICALS

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For many years, lumber producers have been interested in preventing various discolorations in lumber commonly known as sap stain or blue stain. Since most of these discolorations originate from fungi which grow in and on the sapwood during the early stages of the seasoning of the wood, considerable research has been done on the application of chemicals at the sawmill to control these fungi.

From time to time, various methods have been used to determine which chemicals were worthy of large-scale testing on commercial piles of lumber. Almost always the first step has been to determine the fungicidal value of the chemical by making a Petri-dish malt-agar test. Reports on such tests and a description of the methods used are given, for example, in articles by Hatfield (1, 2, 3) and Schmitz (5).

When the Petri-dish tests indicated that a chemical had promise as an antistain chemical, it was next tested under some type of field conditions. One field test method consisted of spraying freshly cut "bolts" of logs with the chemicals to determine whether they would prevent sap stain. It was soon found that this method of evaluation was cumbersome and expensive and gave inconclusive results.

During the summers of 1929 and 1930, preliminary small-scale tests were established at eight sawmills in three southern States, and since then similar small-scale tests have been used in exploratory studies on sap-stain control chemicals. A summary of this work and a description of the methods used are given by Scheffer and Lindgren (4).[3] Briefly, the small-scale test method described therein made use of unseasoned test specimens cut to a given size, dipped in chemicals to be tested, and stacked in boxed-in test piles.

The establishment of small-scale tests with unseasoned wood at the sawmills was a logical development. Such tests had limitations, however; for, since there was little or no chance to control such external factors as temperature, moisture, or variation in site or intensity of inoculum, usually the tests could be conducted only in the warm months of the year. As conditions varied from year to year, the results of any one year's tests could be accepted only as indicative, and large-scale commercial tests were still required to prove the value of a chemical.

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[2] Wyandotte Chemicals Corporation, Wyandotte, Michigan, and Ethyl Corporation, Detroit, Michigan, respectively.

[3] This bulletin also contains numerous references to special articles describing tests that were made and results obtained by the tests.

Between 1929 and 1932 there was a definite change at the sawmills from the use of soda dips to either organic mercury compounds or to highly chlorinated phenols. These new organic compounds had considerably higher fungicidal values than soda, but the use of buffering agents with them seemed advantageous. Over the next few years various chemicals came into use, often in certain combinations employed to reduce skin irritation or for some other reason, but not always with an understanding of the fungicidal values to be realized.

Because of the utility of carbonates and bicarbonates as well as borax in combination with the organic compounds, Monsanto Chemical Company and Wyandotte Chemicals Corporation entered upon a joint research program to gain further information on such combinations. To begin with, a laboratory method was needed, capable of predicting with reasonable accuracy and promptness the possible results of large-scale applications on merchantable lumber.

DESCRIPTION OF THE NEW LABORATORY TEST METHOD

To expedite investigations of potential sap-stain control chemicals, a laboratory method was proposed which would make use of unseasoned (green) wood. Wood discs cut from freshly sawn logs appeared to be suitable for the laboratory tests, and the method finally adopted became known as the "Unseasoned Wood Disc Test Method." This paper will describe the method and compare the results obtained in the laboratory with actual field tests.

Selection of the Wood

Laboratory tests can be made with any species of wood, depending largely on the information desired. In general, fast-growth sapling logs, 3–4 ft. long and 4–6 in. in diameter, are most convenient. Although it is preferable to use the wood immediately after felling, our tests have demonstrated that freshly cut logs can be satisfactorily used some distance from the point of origin if the ends of the logs are coated with hot paraffin or with a paraffin-base paint to prevent excessive evaporation during transit, and the logs are crated to prevent barking during shipment. If desired, logs thus handled can be placed in cold storage at 20° to 30° F. and the general condition of the wood will remain satisfactory for test purposes for as long as several months.

The Incubation Chambers

Before cutting test specimens from the fresh logs, incubation chambers should be assembled. For these chambers, standard 1-qt. wide-mouthed Kerr Mason jars are used, in sufficient number to accommodate the discs to be used in the test, usually 4 discs to a jar. Holes $\frac{1}{2}$ in. in diameter are punched in the flat metal jar lids, and the holes are then plugged with cotton. Three or four short lengths of 10-mm. glass tubing are placed in the bottom of each jar. Round wood applicator sticks are bent to the

breaking point in several places and shaped into a roughly circular outline, to separate each wood disc from the one above or below it in the incubation chamber.

The parts of an assembly and a completed incubation chamber are shown in figure 1, A.

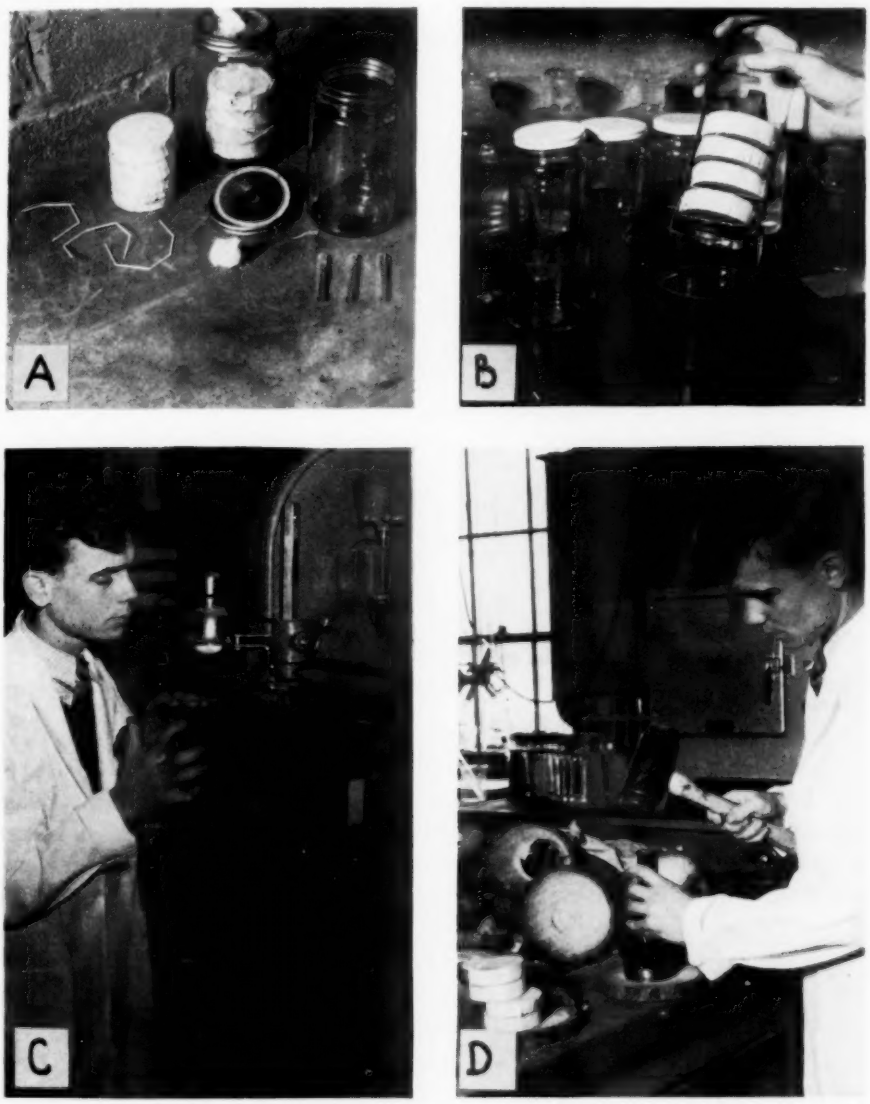

FIG. 1. A. Component parts and complete wood-disc incubation chamber. B. Metal rack for dipping wood discs. C. Preparation of discs with a band saw. D. Cutting discs with an arch punch.

The Dipping Rack

A rack suitable for holding the wood discs while dipping them in the antistain chemicals consists of a U-shaped piece of $\frac{3}{4}$-in. strap copper with

suitable cross-strips riveted to it at proper intervals to support the wood discs. An L-shaped strap extending from the bottom strap and secured along the back of the rack helps keep the discs in place during the dipping operation (Fig. 1, B). A modification of this model makes use of lugs riveted to the copper strap, with a 4-mesh double-crimped copper cloth, of wire size sufficient to give rigidity, soldered to the lugs.

The Dip Solutions

Water solutions containing the desired concentrations of stain-control chemicals are prepared in advance. The wood sections are then dipped immediately after preparation to prevent drying out. One liter of each concentration of each chemical is used for dipping. Four to six discs are dipped in each solution and placed in the Mason jar as described below. At least 20 discs should be used for each chemical or concentration of chemical included in the test.

FIG. 2. Dipped discs in place on glass rods for the 30-min. conditioning period before inoculation.

Preparation of the Wood Discs

A section about 6 to 8 in. long is discarded from the end of a fresh sapling log because of possible desiccation or infiltration of materials from the end coating, and only that portion of the log free from knots and scars is retained. Then, using a band saw, sections about $\frac{1}{2}$ in. thick are cut, and from within the bark of these sections uniform circular discs not over $2\frac{3}{4}$ in. in diameter are punched with an arch punch (Fig. 1, C and D). These punched-out discs are selected from the portion of the rough section containing the greatest amount of springwood and which is most free from heartwood, compression wood, and heavy summer rings.

During the interval between sawing and dipping of the discs, the wood sections are covered with wet towels to prevent desiccation. However, with dip solutions prepared in advance, no longer than an hour need elapse between cutting and dipping.

Dipping and Inoculating

The wood discs are easily and uniformly dipped by placing them in the rack and immersing them in the antistain solution for 15 seconds. After

dipping, the discs are laid edgewise on glass rods for 30 min. to allow the solution to soak into the wood and the excess to drip off (Fig. 2). Test discs from three or more different logs are used for each concentration of chemical tested in order to reveal any differences in sap-stain susceptibility that might occur in individual trees.

The discs are next sprayed with a spore-mycelium suspension of an individual sap-stain organism or with a mixture of organisms. In general, it will be found that if a spore-mycelium suspension of *Ceratostomella pilifera* (or any other individual sap-stain organism) is made, it is unnecessary to add the spores of such molds as *Aspergillus* sp., *Penicillium* sp., or *Trichoderma* sp., for mold spores are generally present on the wood in sufficient numbers to assure inoculation. Usually, about 50 discs can be inoculated with 100 ml. of inocula suspension.

Inoculation

Immediately after spraying, four of the discs are tiered one above another in the incubation jars on the 10-mm. glass tubing, separated by the bent applicator sticks. The punched metal lid containing the cotton vent is then fixed in place by screwing on the metal ring. The vent allows volatile solvents or extractives as well as staling products to escape from the jar and fresh air to enter. The jars or chambers are then placed in a constant temperature room or incubator maintained at approximately 80° F. and approximately 75 per cent relative humidity. In general, the wood discs contain sufficient moisture to keep the jars humid throughout the test, but a small amount of water can be added in the bottom of the jar if the jar appears to be drying out.

Examination

The wood discs should be examined after 1, 2, and 4 weeks, and after longer periods if desired. The nature of the fungus growth on the discs can be recorded; and, if desired, the degree of stain can be accurately determined by the use of a transparent counting card with Jeffer or other suitable ruling thereon.

RESULTS OF LABORATORY TESTS

In one experiment, the stain-inhibiting qualities of ten formulations (Table 1) were compared, using the sap-stain organism *Ceratostomella pilifera* on wood discs prepared from shortleaf pine (*Pinus echinata*). Readings were made at weekly intervals for 110 days. It was concluded that 3 weeks' incubation gave most reliable and most reproducible results; if the tests extended beyond that time, contaminants often obscured the actual blue stain growth, for no attempt is made to work under aseptic conditions.

In figure 3, photographed after 110 days' incubation, the treated blocks on the left are "bright" (unstained), whereas the untreated specimens on the right are badly discolored by stain fungi.

TABLE 1.—*Identification and composition of products*

Product	Percentage by weight and approximate composition
A[a]	20.0 Sodium pentachlorophenate (Tech.) 30.0 Modified soda (hydrate) 50.0 Borax (hydrate)
B	96.0 Sodium pentachlorophenate 4.0 Sodium carbonate
C	51.0 Sodium tetrachlorophenate 46.0 Sodium 2-chloro-o-phenylphenate 3.0 Water
D	16.0 Sodium pentachlorophenate 77.0 Sodium tetrachlorophenate 7.0 Water
E	92.0 Sodium pentachlorophenate 4.0 Sodium hydroxide 4.0 Water
F	6.4 Ethyl mercury phosphate 72.4 Borax (hydrate) 19.5 Kaolin or talc 1.7 Sodium carbonate
G	70.0 Sodium o-phenylphenate 6.3 Sodium hydroxide 0.4 Sodium carbonate 21.3 Water
H	Technical sodium beta-naphthalate
I	73.8 Sodium 2-chloro-o-phenylphenate 8.8 Sodium hydroxide 2.4 Sodium carbonate 15.0 Water
J	89.8 Sodium tetrachlorophenate 4.8 Sodium hydroxide 0.4 Sodium carbonate 5.0 Water

[a] U. S. Patent 2,392,987, January 15, 1946, covers compositions of this type.

Results from this experiment after 1 and 3 weeks' incubation are given in table 2. These figures do not, however, tell the whole story. Observations were made on the rate of growth of organisms as well as on the percentage of stain produced. Stain was visible on the control blocks 2 or 3 days after inoculation, and the rate of growth of the fungus was rapid until the blocks became completely stained. By the end of 4 or 5 days the blocks were usually 100 per cent stained. In comparison to the controls, product A at 0.75 per cent concentration inhibited the initial rate of growth of *Ceratostomella* for several days, but after the fungus began to develop, the rate of growth was rapid. At 1.5 per cent, however, the rate of growth was measurably inhibited. In the case of a 2.5 per cent concentration, the fungus did not begin to develop until after 14 days and even then its rate of growth was not rapid. At 3.5 per cent, the organism was completely killed.

At 0.75 per cent concentration product B was more successful in retarding the growth rate of the fungus than product A, but at each successively higher concentration it became relatively less effective, until at the

1.5 per cent concentration it was practically identical with product A, at 2.5 per cent it was inferior to product A, and at 3.5 per cent it still allowed the test organism to grow.

TABLE 2.—*Results of stain tests on shortleaf pine blocks after 1 and 3 weeks' incubation*

Product	Percentage of block area stained after time indicated, at a concentration of:							
	0.75 per cent		1.5 per cent		2.5 per cent		3.5 per cent	
	1 wk.	3 wks.	1 wk.	3 wks.	1 wk.	3 wks.	1 wk.	3 wks.
A	88	100	8	41	0	2	0	0
B	30	84	12	47	0	7	0	2
C	75	100	5	52	0	19	0	3
D	29	80	10	30	0	7	0	1
E	19	90	6	54	0	11	0	2
F[a]								
G	.....	.....	.....	.....	34	99	2	91
H	.....	.....	.....	.....	56	92	.....	.....
I	.....	.....	.....	.....	55	98	36	92
J	.....	.....	.....	.....	0	5	0	0
Borax	.....	.....	.....	.....	0	5	0[b]	11[b]
Untreated control	100	100	100	100	100	100	100	100

[a] Blue stain observations could not be made for product F because of heavy contaminants.

[b] The concentration was 5.0 per cent.

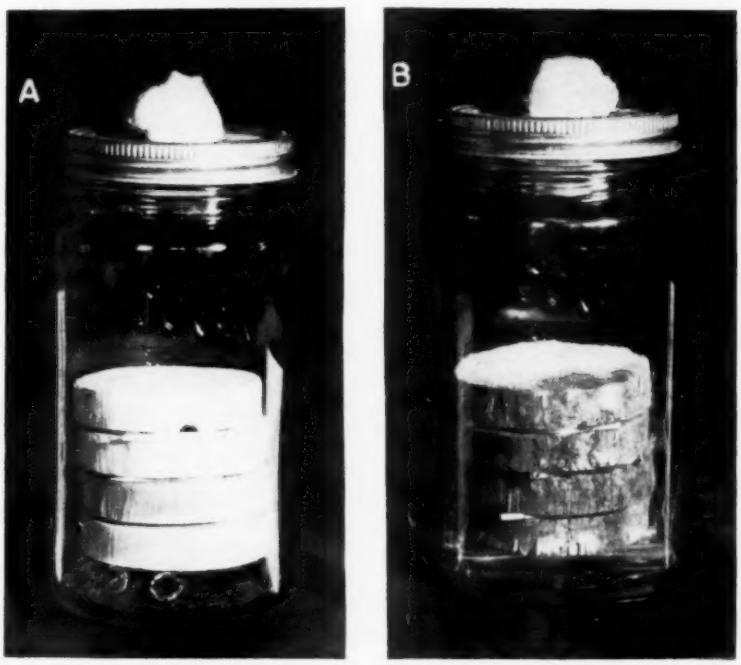

FIG. 3. Wood discs after 110 days' incubation. Treated blocks (A) are "bright" (unstained); untreated specimens (B) are badly discolored by stain fungi.

MILL TESTS

In order to compare laboratory results with field results, several tests were set up at lumber mills in various sections of the United States. Since conditions were purposely made more severe in laboratory tests than would be expected in the field, it was anticipated that lower concentrations of chemicals could be used in the field. All trials were carried out under conditions as carefully controlled as was practical for work of this kind. Results obtained from one of the most extensive series of tests on pine lumber, conducted at the Southern Lumber Company, Warren, Arkansas, are presented. Although several chemicals were tested at this plant, for comparative purposes only products A and B (Table 1) are included.

The lumber used was native shortleaf and longleaf Arkansas pine, most of it having a high percentage of sapwood. It was sawn from logs that had been felled 3 or 4 days previously. Approximately 1000 board feet of lumber was included in each test on each chemical and at each concentration, and about 200 board feet of undipped lumber was scattered throughout the stack as a control. All the lumber was 1 or $1\frac{1}{2}$ in. thick and 16 ft. long. The width varied from 4 to 12 in., averaging 6 to 8 in.

All the dipping was done by hand for the sake of more accurate control of the dipping time and other variables. The concentration of each chemical tested was determined by titrating with 0.1 N sulfuric acid to the bromphenol blue end point. Before making this field test, titrations had been made in the laboratory on solutions of known concentrations in order to plot calibration curves, so that the concentration of the solution could be accurately determined in this manner in the field.

The lumber was hand-dipped for exactly 15 seconds, drained for a few seconds, and then bulk-piled for 1 to 2 days, each piece being marked on the end with colored keel. This bulking corresponds to prevalent mill practice. After this period of bulk-piling the lumber was stacked in the drying yard in the usual manner except that no space was left between boards in the courses. This greatly reduced the amount of air circulation and slowed the drying of the lumber considerably, giving the stain a greater chance to develop.

The dipping tests were started on September 12 and the inspection was completed on the following January 10, a total seasoning period of approximately 115 days. The weather during this time was very damp and the temperature comparatively high. Therefore, it is thought that the test was relatively severe for this locality. This is borne out by the fact that the amount of stain on the untreated lumber was unusually high for most of the test pieces.

After seasoning, the lumber was removed from the stack one piece at a time, and the percentage area of sapwood estimated. Since the amount of sapwood in any given board is somewhat irregular and is different on each side of the board, the accuracy of this estimate was probably not better than $\pm$ 15 per cent. Next, both the top and bottom of each board was inspected

to determine the percentage area of the total board which had been stained. Again this estimation was rough inasmuch as the stained areas are sometimes spotted irregularly over the entire board. However, it is believed that this estimate was accurate to within ± 10 per cent.

The results obtained on pine tested at the Southern Lumber Company appear in table 3 and in figure 4. The figures listed in column C of table 3 will be of most interest to the lumber mill manager or superintendent, for they represent the amount of stain which he would have in his yard. However, the figures in column F, which are the percentages of the total sapwood area stained, are the most valuable from a theoretical point of view, inasmuch as only the sapwood will stain and comparative results with different chemicals can be obtained only by basing results on the amount of sapwood stained

TABLE 3.—*Results of field stain tests on southern pine, Southern Lumber Company, Warren, Arkansas*

Chemical and concentration, in lb. per 100 gal.	Boards showing some stain	Average percentage of total area stained		Sapwood area on stained boards	Average percentage of sapwood area stained	
		Stained boards	All boards		Stained boards	All boards
	A	B	C[a]	D	E[b]	F[c]
	Pct.			*Pct.*		
Product A, 7.67	15	7	1	64	11	2
Untreated control	66	51	34	66	77	52
Product A, 9.16	16	13	2	65	20	3
Untreated control	87	54	47	62	87	76
Product A, 12.92	0	0	0	60	0	0
Untreated control	85	43	37	52	83	71
Product B, 8	30	10	3	61	16	5
Untreated control	60	53	32	67	79	48
Product B, 10	19	14	3	63	22	4
Untreated control	94	49	46	68	72	68
Product B, 14	10	6	1	56	11	2
Untreated control	85	43	37	52	83	71

[a] $C = \frac{B \times A}{100}$

[b] $E = \frac{B}{D} \times 100$

[c] $F = \frac{C}{D} \times 100$

rather than upon the total area stained. Listed directly under each chemical at each concentration tested are the same data for the untreated portions of each test stack closest to the test lumber described above it. It can be seen from this table (Column F) that the use of a suitable chemical treatment will reduce the percentage of sapwood stained from a maximum of 76 per cent to a negligible amount.

CONCLUSION

Laboratory and sawmill application studies have demonstrated that there is good correlation between results obtained in the laboratory with the un-

seasoned wood disc method for evaluating sap-stain control chemicals and the results obtained in sawmill tests. This new laboratory test method is

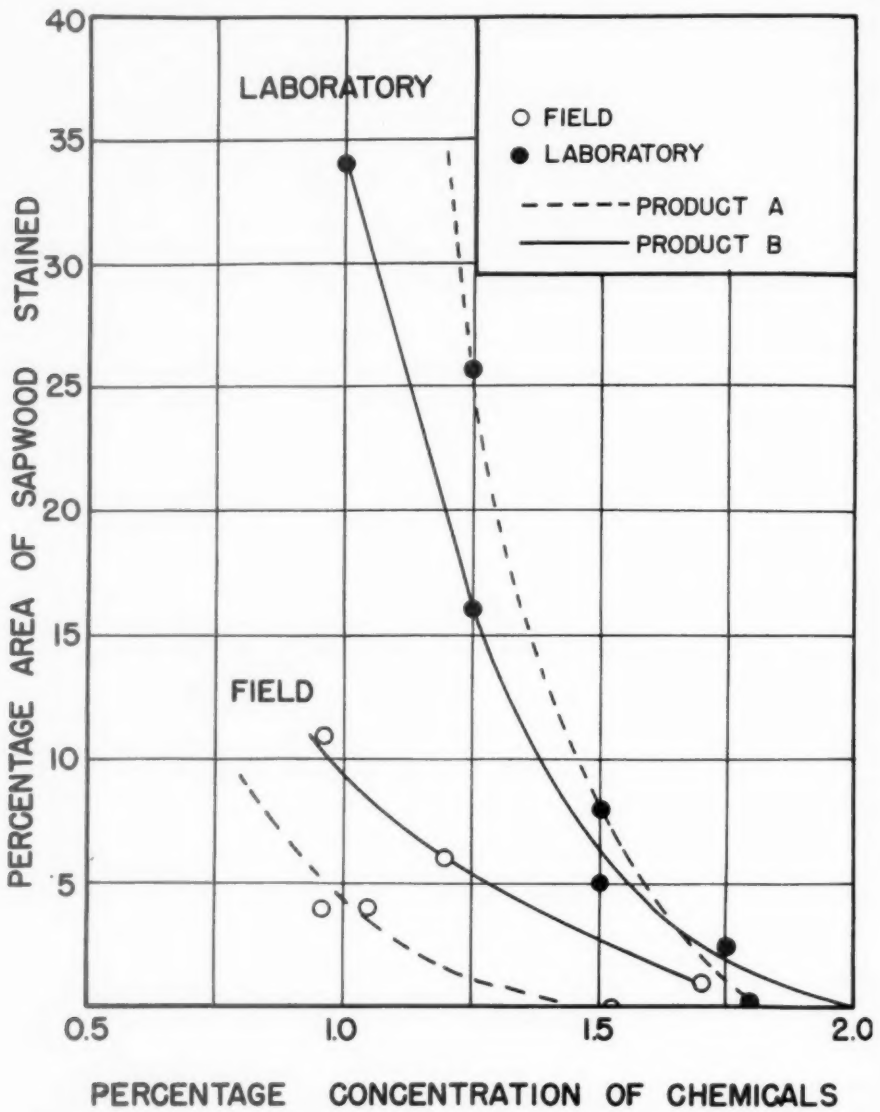

FIG. 4. Comparison of laboratory and field sap-stain control tests on Arkansas pine.

more economical of time than are former methods, and it should be useful to technicians studying antistain chemicals and their efficacy.

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LITERATURE CITED

1. HATFIELD, IRA. Recent experiments with chemicals suggested for wood preservation. Proc. Amer. Wood-Pres. Assoc. **27**: 304–315. 1931.
2. ————————. Further experiments with chemicals suggested as possible wood preservatives. Proc. Amer. Wood-Pres. Assoc. **28**: 330–339. 1932.
3. ————————. Toxicity in relation to the position and number of chlorine atoms in certain chlorinated benzene derivatives. Proc. Amer. Wood-Pres. Assoc. **31**: 57–66. 1935.
4. SCHEFFER, THEODORE C., and RALPH LINDGREN. Stains of sapwood and sapwood products and their control. U. S. Dept. Agr. Tech. Bul. 714. 1940.
5. SCHMITZ, HENRY, ET AL. (Schmitz acted as secretary for a group of 15 scientific workers.) A suggested toximetric method for wood preservatives. Ind. Eng. Chem. Analyt. Ed. **2**: 361–363. 1930.

FUSARIUM STRAINS CAUSING PEA AND BEAN ROOT ROT[1]

OTTO A. REINKING

(Accepted for publication January 14, 1950)

INTRODUCTION

Studies started in 1937 of the fusaria causing root rot of peas indicated that there are differences in strains of the pea pathogen, *Fusarium solani* v. *martii* f. *2*. These appeared to vary in degree of pathogenicity, morphology, and cultural characters. The first tests with pea strains, run in 1939 to determine their pathogenicity, were made in nutrient solution cultures (8). All proved to be true pea root-rot organisms. The tests served the purpose of identifying the fusaria that produce severe root rot. Since nutrient culture tests may bring to mind several variables, further tests were undertaken in pots of soil with controlled temperatures and soil moisture. Preliminary tests were run in 1939 in so-called "climate chambers" in which the temperatures could be regulated and the soil moisture held constant by the use of auto-irrigation pots (8). The soil in the pots was infested with a given amount of a cornmeal-sand culture, and the checks with the same amount of sterile cornmeal-sand. In addition to proving the pathogenicity of the Fusarium strains in soil cultures, corroborating the nutrient culture tests, the ability of some strains to produce seed decay was demonstrated. Two strains produced severe seed decay; two others did not have this ability to the same degree. The tests indicated that a possible difference in virulence existed between strains; one with a root-rot index of 89 consistently caused more root rot than another with an index of 72. Variability in strains of pathogens is nothing new; it has been shown to be true with a number of pathogenic fusaria (7, 10, 12, 13, 14).

On July 19, 1940, a Fusarium was isolated from the reddened root of a California-grown red kidney bean from one of the bottomland farms, where pea root rot is general, in the Genesee River Valley near Mount Morris, New York. Preliminary inoculations with this organism indicated that it apparently was only a weak bean parasite, but a virulent pea root-rot parasite. These findings brought up the whole question of pathogenic strains; whether certain strains of the pea root-rot Fusarium might be true parasites or semi-parasites on bean, and certain strains of the bean root-rot Fusarium might be parasitic or semi-parasitic on peas. With these questions in mind, along with an over-all study of the fusarial pea root-rot organisms, further isolations of fusaria from pea and bean were made for host and cross-inocula-

[1] Journal Paper No. 802, New York State Agricultural Experiment Station, Geneva, New York, July 27, 1949.

The writer wishes to acknowledge aid with some of the inoculation investigations to Drs. R. F. Suit and R. O. Magie, formerly of the Division of Plant Pathology of the Geneva Station, and to Dr. W. T. Schroeder, a member of the Division.

tion studies. A number of closely allied fusaria, which from morphological and cultural characters appeared not to fall in the pathogenic group, were included to serve as checks in the pathogenicity studies.

METHODS

Studies were made on the morphology and cultural characters of strains isolated from peas and beans. All cultures used were obtained from single spores of each strain. The source of strains is given in table 1.

TABLE 1.—*Source of Fusarium strains used in studies*

Pea Fusarium strains

1000—California red kidney bean, Mount Morris, N. Y. From weakened plant growing on edge of low, poorly drained spot in field. Plant with yellowed leaves, poor root system, and red-brown taproot. July 19, 1940.
1001—Early Wales pea grown in pea variety test at Mount Morris, N. Y. July 17, 1940.
1002 and 1003—Surprise pea collected from fertilizer test planting at the Canning Crops Farm, Geneva, N. Y. June 14, 1940.
1004—Surprise pea grown in a pea variety test planting at Junius, N. Y. Little root rot present. July 16, 1940.
1005—Thomas Laxton pea grown in a 2-acre field planting at the Canning Crops Farm, Geneva, N. Y. June 13, 1940.

Bean Fusarium strains[a]

1032, 1033, and 1034—Mature California red kidney bean from Plant Disease Garden, Geneva, N. Y. Plants apparently produced a normal crop. October 30, 1943.
1035—Mature California red kidney bean grown in experimental plots, Geneva, N. Y. Plants apparently produced a normal crop. November 1, 1943.
1036—Mature California red kidney bean, Hall, N. Y. Plants apparently produced a normal crop. November 1, 1943.
1037 and 1038—Mature California red kidney bean, Markham Road, Town of Geneva, N. Y. Plant was stunted and had a badly rotted root system as well as a red-brown foot and taproot. November 1, 1943.
1039—Mature Fordhook bush Lima bean, Geneva, N. Y. November 6, 1943.
1040—Mature Blue Lake pole bean, Geneva, N. Y. November 6, 1943.

[a] Each strain was isolated from the cortical layer just under the browned tissue of the taproot.

The pathogenicity studies were divided into separate categories as follows: inoculations with pea Fusarium strains on peas and beans growing in soil infested with cornmeal-sand inoculum; inoculations with bean Fusarium strains on beans and peas growing in soil infested with cornmeal-sand inoculum and with standard liquid spore suspensions, and growing in sand nutrient cultures infested with standard liquid spore suspensions; final inoculations with pea and bean Fusarium strains on peas and beans in sand nutrient cultures with standard liquid spore suspensions; and soil inoculations with bean roots that had been infested with pea Fusarium strains. In the soil inoculation tests using cornmeal-sand cultures, there was a possibility that organic decomposition products from the addition of such inoculums may have caused discoloration of the foot and roots of plants under test (4). To eliminate this factor, the last tests were run with plants growing in soil and sand nutrient cultures using a standard liquid spore suspension of the organisms for inoculation.

As a measure of infection, a root-rot index was set up based on an estimate of severity of rotting. The following ratings for infection were used in all instances: severe, 100; moderate, 67; slight, 33; and healthy, 0.

MORPHOLOGY

Spore Measurements

A morphological study of various strains showed that there was a consistent mean difference in measurements between the pea (*F. solani* v. *martii* f. *2*) and bean (*F. solani* v. *martii* f. *3*) pathogens (Fig. 1). There was a

TABLE 2.—*Spore measurements of Fusarium strains isolated from peas and beans*

Pea root-rot strains
F. solani v. *martii* f. *2*. Type. 38 × 4.8 μ (30–46 × 3.9–5.0) (20–58 × 3.5–5.5) and related strains
Virulent strains
1002—37 × 5.0 μ (33–46 × 4.5–5.5) 82 per cent 3-septate, few 4-septate, 5 per cent
Moderately virulent strains
1000—38 × 5.0 μ (31–51 × 4.5–6.0) 82 per cent 3-septate, few 4-septate
1001—38 × 5.0 μ (26–40 × 4.5–5.5) 91 per cent 3-septate, few 4-septate
Saprophytic or slightly parasitic strains
1003—36 × 5.4 μ (30–40 × 5.0–6.0) 86 per cent 3-septate, few 4-septate
1004—38 × 5.8 μ (29–44 × 5.0–6.7) 88 per cent 3-septate, few 4- and 5-septate
1005—37 × 5.4 μ (31–45 × 4.5–6.0) 91 per cent 3-septate, 4- and 5-septate, occasionally 59 per cent and 3 per cent
Bean root-rot strains
F. solani v. *martii* f. *3*. Type. 44 × 5.0 μ (33–52 × 4.5–5.5) and related strains
Virulent strains
1033—41 × 5.5 μ (33–48 × 4.8–6.0) 95 per cent 3-septate, occasional 4-septate
1034—40 × 5.1 μ (33–47 × 4.5–6.0) 75 per-cent 3-septate, occasional 4- and 5-septate
1038—44 × 5.1 μ (33–54 × 4.2–6.0) 88 per cent 3-septate, some 4-septate up to 10 per cent
Saprophytic or slightly parasitic strains
1032—38 × 5.0 μ (29–48 × 4.2–5.7) 95 per cent 3-septate, some 4-septate
1035—39 × 4.9 μ (30–53 × 4.4–5.2) 80 per cent 3-septate, few 4-septate and 5-septate
1036—36 × 5.1 μ (29–42 × 4.2–5.4) 97 per cent 3-septate, some 4-septate
1039—34 × 4.9 μ (24–42 × 4.5–5.7) 96 per cent 3-septate, some 4 septate
Saprophytic strains
1037—41 × 5.1 μ (33–48 × 4.5–5.7) 96 per cent 3-septate, some 4-septate
1040—36 × 4.8 μ (29–42 × 4.2–5.3) 91 per cent 3-septate, some 4-septate

rather wide range in measurements within each group and apparently an overlapping of the strains, if one may judge from the average measurements of spores taken from normal cultures. All spore measurements were made from single-spore cultures growing on 2.5 per cent potato-dextrose agar in the light of an ordinary laboratory room and of spores taken from pionnotes masses from cultures that were regarded as being in so-called "high culture." No attempt was made to determine the possible range of each strain. Such differences occurring in fusaria have been reported in detail by others (5, 10, 12, 13, 14), and were found in all strains studied in the present investigations. On woody tissues, such as white sweet-clover stems and elm twigs, the strains studied had a tendency to produce more 4- and 5-septate spores even though the majority were consistently 3-septate. The morphological studies are presented primarily to show that there seems to

be a rather consistent range for both the pea and bean strains made from normal cultures, but that there may be an overlapping of strains. It was thought this might explain the presence of a pea pathogen on bean, indicating that there might be strains that could attack both peas and beans. Spore measurements of the various strains of pea and bean organisms are in table 2. The strains studied are grouped into virulent, moderately virulent, saprophytic or slightly parasitic, and saprophytic strains.

Measurements of the typical 3-septate spores of the virulent pea strains most consistently fell into a class a little shorter and broader than what is considered to be the type for this strain (15, 16) (Fig. 1, A, B). The

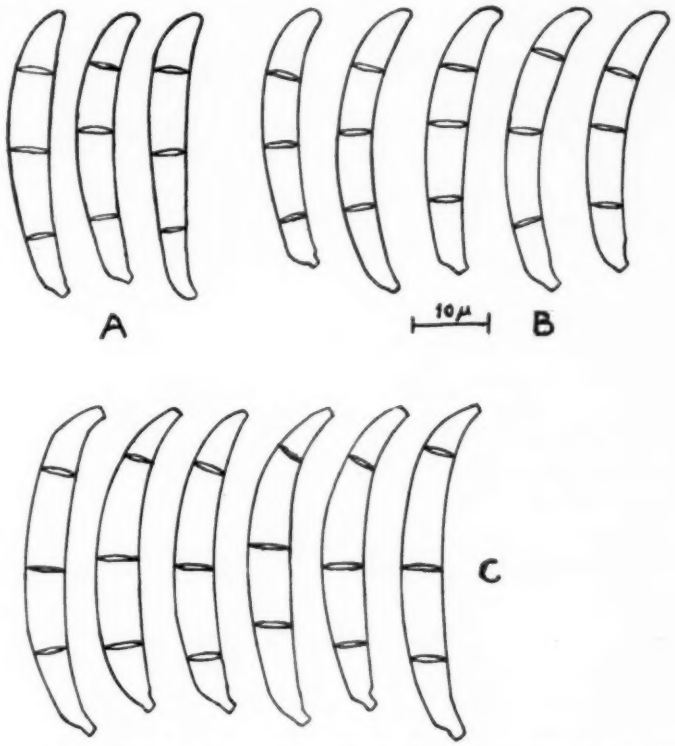

FIG. 1. Typical spores of the pea *Fusarium solani* v. *martii* f. *2* and the bean *Fusarium solani* v. *martii* f. *3* foot-rot and root-rot organisms. Spores from dense pionnotes after 1 month's growth on hard potato-dextrose agar. A. Pea strain, 1000. B. Pea strain, 1002. C. Bean strain, 1038.

measurements of the described type are $38 \times 4.8\ \mu$ $(30\text{–}46 \times 3.9 = 5.0)$ $(20\text{–}58 \times 3.5 = 5.5)$. Those strains classed as moderately virulent fell pretty well into the type but were slightly broader. Strain 1005, classed as a saprophytic or slightly parasitic organism, produced broader spores in proportion to the length and ran rather consistently with more 4- and 5-septate spores on all types of media. Saprophytic or slightly parasitic strain 1004, while closely related to the parasitic strains, normally had broader

spores. The saprophytic or slightly parasitic strain 1003 was consistently broader in proportion to its length than the true parasites. These studies showed that it was not always possible to select parasites from saprophytes by morphology alone and that infection studies may be required to prove this point.

Strains 1032 to 1040 inclusive isolated from beans include virulent bean foot-rot and root-rot parasites as well as mildly parasitic and saprophytic strains. The spore measurements of the virulent, true bean root-rot strains 1033, 1034, and 1038 fell rather closely in the type class for *F. solani* v. *martii* f. *3* (3, 15, 16) (Fig. 1, C). The measurements of the described type are $44 \times 5.0\,\mu$ ($33\text{–}52 \times 4.5 = 5.5$). The spores measured a little broader than that reported for the type. There were, of course, variations as indicated in the range for length and width. The majority of the spores are 3-septate. All strains produced some 4-septate spores and occasional 5-septate spores on hard potato-dextrose agar. On woody tissue cultures, the tendency was to produce more 4- and 5-septate spores. The strains classed as saprophytic or slightly parasitic were shorter than the type. These, according to the inoculation studies, might even have been classed as doubtful parasites. The saprophytic strain 1037 fell within the range of the parasites. In this instance, it was impossible to separate all parasites from saprophytes by purely morphological characters. The short-spore strain 1040, however, consistently showed morphological and physiological differences which from these factors alone placed it out of the bean parasite group.

Culture Characters

Spore measurement studies, along with pathogenicity tests, showed that there are a number of strains of both the pea and bean foot-rot and root-rot fusaria that vary as to pathogenicity as well as morphology. The following review of culture studies will show that they vary in growth characters as well. This variability is nothing new; it has been found with other parasitic fusaria (7, 13, 14). As with size of spores, there may be a variation in growth characters of strains when grown at different times under uncontrolled conditions. Normal cultures of the pea and bean strains produced dense pionnotes masses of spores on hard potato-dextrose agar. Scant mycelium was produced in culture when in this "high" state of growth. All strains could revert to mycelial types, but the tendency is to produce sporodochia or pionnotes spore masses.

The spore masses in general for all pathogenic pea strains were blue-green. Some pathogenic strains, such as 1000 and 1001, generally produced green and buff-colored sporodochial or pionnotes spore masses. As a matter of fact, this characteristic might be produced at times by all of the pathogenic strains. The coloration of agar also varied: generally no color was present, but a wine-red color often was produced by certain strains. It should be noted that the saprophytic or slightly parasitic strain 1005 consistently produced lighter colored pionnotes masses of an olive and buff

and a wine-red agar. Saprophytic or slightly parasitic strains 1003 and 1004 produced cream-buff pionnotes rather consistently, but at times light green and buff spore masses. The agar upon which these two strains grew was never observed to become wine-red. Growth and color may be variable factors affected by environmental conditions (5, 7, 12, 17) and may not serve

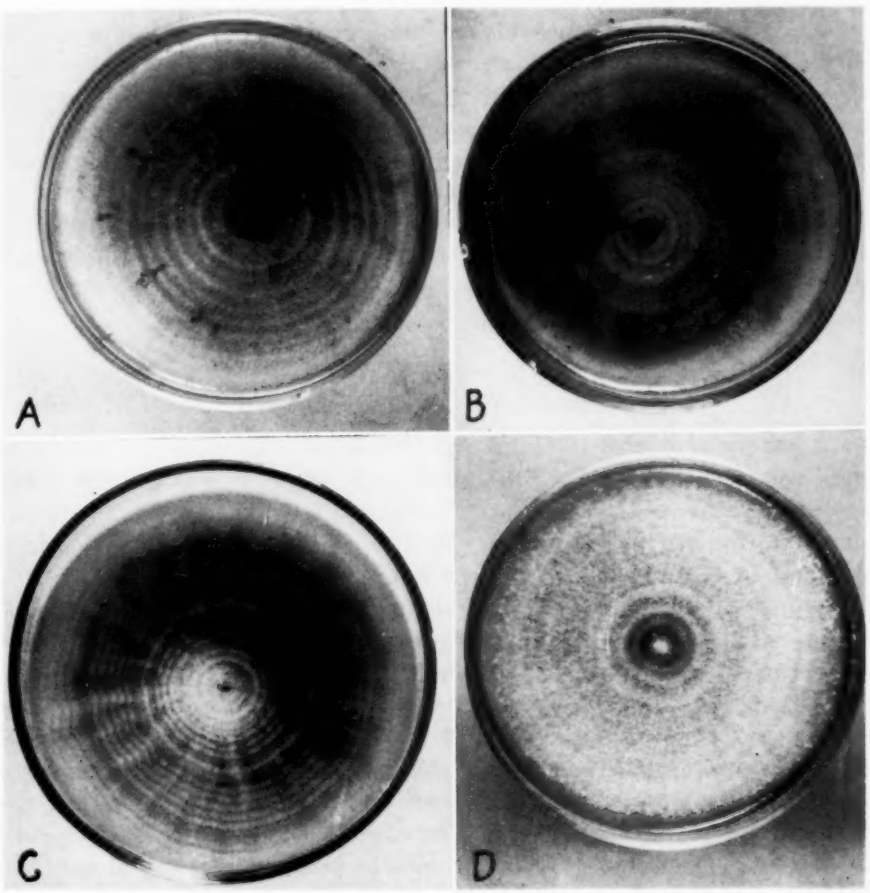

FIG. 2. Culture characters of pea and bean foot-rot and root-rot Fusarium strains. One-month-old cultures growing on 2 per cent dextrose-potato agar. A. Typical virulent pea root-rot strain 1002, showing sectoring. (Photographed March 28, 1941.) B. Moderately virulent pea root-rot strain 1000, isolated from the root of a diseased California red kidney bean. Sectoring culture. (Photographed March 28, 1941.) C. Typical virulent bean root-rot strain 1038. (Photographed December 16, 1948.) D. Saprophytic or slightly parasitic pea root-rot strain 1003. (Photographed March 28, 1941.)

to separate different organisms. It is believed that these characters, along with morphological differences, often may help in differentiating some of the parasitic and saprophytic strains. Growth characters as produced in plate cultures (Fig. 2, A, B, D) seemed to help separate some strains.

All bean strains, whether parasitic or saprophytic, produced blue-green pionnotes masses, except saprophytic strain 1040 which was cream-buff.

Agar was colored wine-red at times by most of the strains, but less often by the highly parasitic strains 1033, 1034, and 1038. The saprophytic strains 1037 and 1040 were not observed to produce a coloration in the agar. Saprophytic strain 1040 in plate cultures grew much faster than the parasitic strains 1033, 1034, and 1038, all of which had an equal rate of growth (Fig. 2, C).

An indication of an unstable condition evidenced by sectoring was observed in some of the pea strains, notably strains 1000 and 1002 (Fig. 2, A, B). Such sectoring occurred in single-spore cultures and is not in these instances a result of mixed cultures (1). This characteristic has been reported previously for various organisms (1, 9, 12, 17). In the writer's experience with Fusarium, it has been frequently observed, especially in the Martiella section and also in other sections (9). It was suspected that this sectoring might have a bearing on production of parasitic strains which varied in pathogenicity both to peas and beans, but much more work must be done to determine such a point.

PATHOGENICITY STUDIES

Inoculations with Pea Fusarium Strains

A history of strains 1000 through 1005 is given in table 1. All strains were isolated from peas, with the exception of 1000 which was isolated from a diseased bean. Strain 1000 had morphological and cultural characters similar to the pea strains and was used as a key organism to demonstrate whether, in the greenhouse, a Fusarium strain isolated from bean could go to peas. Pea strains 1001 through 1005 were selected by morphological and growth characters as possible representatives of pea pathogens and as organisms closely related but possible saprophytes.

Pea inoculations in soil with cornmeal-sand inoculum. On December 20, 1940, three pots of soil were infested with 2 tablespoonfuls of a cornmeal-sand culture of each strain and each pot was planted with 10 Thomas Laxton pea seeds. Nine pots of noninfested soil were used as checks for the series and each was planted with the same number of seeds. All pots were placed in moistened sphagnum moss on a greenhouse bench and separated in groups according to the different fungus strains. The day temperature in the greenhouse fluctuated during the test between 69° and 73° F. The soil in the pots was kept rather dry to prevent seed decay. Data were taken on stand, number of days to severe disease appearance, index of root-rot, and average length of plants. The appearance of severe disease determined the date of removal of plants and a possible difference in virulence of strains. Positive reisolations were made from all diseased plants. The results of this test are in table 3.

The strains are listed in order of virulence as indicated by the data obtained. Strain 1002 proved to be the most virulent. Severe stunting and wilting with death of some plants resulted 21 days after planting (Fig. 3, A). Plants showed severe foot-rot and root-rot typical of that for the Fusarium

type, with a root-rot index of 100. The cotyledons of the diseased plants were badly decayed, while those of the check plants were still firm and healthy. Infection seemed to extend from the seed upward into the stem or foot and downward into the root system. Because of severe root-rot, very little secondary root development was evident. Plant measurements showed a pronounced stunting when compared with healthy check (Fig. 3, C).

TABLE 3.—*Tests with strains of Fusarium isolated from peas and bean inoculated on peas*[a]

Strain	Stand, per cent	Date dug (1941)	Wilting and dying, days after planting	Root-rot index	Av. length of plants, inches
1002	97	Jan. 10	21[b]	100	7
Check	100	do	None	0	10
1000	97	Jan. 18	29[c]	67	11
1001	100	do	29	90	11
Check	100	do	None	0	14
1005	100	Feb. 5	None[d]	33	14
1004	100	do	None[d]	10	16
1003	97	do	None[d]	0	17
Check	93	do	None[e]	0	18

[a] Ten Thomas Laxton peas in each pot; three pots to a series. Strains listed according to degree of virulence. Planted Dec. 20, 1940, in sterilized soil.
[b] Plants severely wilted and stunted.
[c] Plants with some wilting and stunting.
[d] Plants with no wilting but some stunting.
[e] Plants normal.

The next plants to show severe wilting and marked stunting were those in pots infested with strains 1000 and 1001 (Fig. 3, B). The severe disease appeared 8 days later than that of the series inoculated with strain 1002. Both strains had a marked similarity in growth and morphological characters. The plants in this series were badly stunted and wilted at the time of digging, 29 days after planting. At that time they had a root-rot index of 67 and 90, respectively. Some secondary roots had started to develop, especially in the plants in pots infested with strain 1001. Strain 1000 isolated from foot rot of red kidney beans proved to be virulent on pea.

Plants growing in pots infested with strain 1005, while definitely stunted, did not show any other abnormal development of the tops of plants up to the time they were dug, 47 days after planting. At this time, pea pods were developing on the vines. All plants, however, were slightly affected with foot rot and root rot, with an average disease index of 33, and had few secondary roots. Strain 1005 differed in culture characters from the other strains in this series. It apparently is a slightly virulent strain, or might even be classed as a saprophyte.

Culture 1004 isolated from diseased peas was a borderline strain of *Fusarium solani* v. *martii* f. *2*, if we may judge from its morphological and physiological characteristics. Infected plants were stunted, with otherwise healthy tops at the time of digging, 47 days after planting. Foot-rot and

root-rot development was very slight with a root-rot index of only 10. The strain apparently is a saprophyte or at most a very weak parasite.

Strain 1003 isolated from a diseased Surprise pea from the same field in Geneva from which strain 1002 was isolated had growth characters somewhat different but closely resembling those of the other strains. Morphological studies showed a distinct variation from the pathogenic types. Similar strains are frequently isolated from diseased plants and without

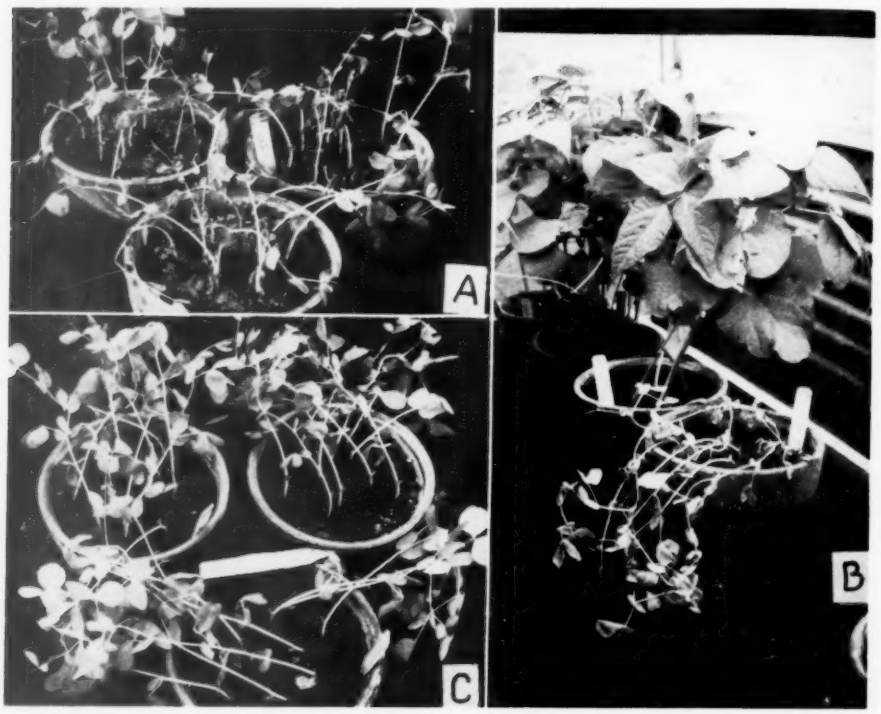

FIG. 3. Soil inoculation series with pea Fusarium strains (December 20, 1940). A. Severe stunting and wilting of pea plants 21 days after planting in soil infested with virulent pea strain 1002. Root-rot index 100. (Photographed January 7, 1941.) B. Pea plants with wilting and stunting that first appeared 29 days after planting in soil infested with moderately virulent pea strain 1000, isolated from a field of diseased California red kidney beans. California red kidney beans in adjoining pots not affected. (Photographed January 17, 1941.) C. Healthy check plants. (Photographed January 9, 1941.)

detailed study could be mistaken for pathogenic forms. The infection tests indicated that strain 1003 was nonparasitic to peas since no real foot rot or root rot developed, but there apparently was some stunting of the plants growing in infested soil.

Bean inoculations in soil with cornmeal-sand inoculum. Strains 1000, 1001, and 1003 were tested as to their pathogenicity on beans. On December 20, 1940, ten Jumbo Marrow bean seeds were planted in each of three pots of autoclaved soil which had been infested with a cornmeal-sand culture of one of the three strains. The results of the tests are in table 4.

TABLE 4.—*Tests with strains of Fusarium isolated from peas and beans and inoculated on Jumbo Marrow bean*

Strain	Source	Stand, per cent	Root-rot index	Average length of plants, inches
1000	Bean	73	29[a]	16
1001	Pea	87	31[a]	16
1003	Pea	87	0	17
Control		90	0	18

[a] Streaks or flecks on foot or taproot.

Strain 1000, isolated from bean, and strain 1001, isolated from pea, produced a slight streaking and flecking on the bean taproot with a root-rot index of 29 and 31, respectively, which apparently was reflected in a slight stunting as indicated in the growth measurements. These tests indicate that pea strains could cause streaking and flecking on bean roots along with an apparent slight stunting of growth, but were not virulent bean parasites.

To test the strains further on beans, the infested soils from the series of pea inoculations were replanted with beans to determine further whether or not the pea strains and strain 1000 isolated from bean could infest beans. Instead of using one bean variety, each of the 3-pot series was planted with 10 seeds of California-grown red kidney, Yellow Eye, and Jumbo Marrow beans. Because the original pea tests were harvested at different dates according to degree of strain virulence, the bean tests were planted separately as soon as the pea tests had been completed. The results of these investigations are in table 5, column 1.

The test with strain 1002 planted on January 22, 1941, and dug on February 4, 1941, at which time pods 4 in. long had developed, showed that the fungus did produce a slight flecking on all varieties of beans, with a root-rot index for infection of 11 on California-grown red kidney, 29 on Yellow Eye, and 33 on Jumbo Marrow, or an average index of 24 (Table 5, column 1). An occasional Jumbo Marrow plant was severely infested. No significant difference was noted in growth measurements. These data would indicate slight pathogenicity of pea strain 1002 on beans.

The second lot of pea test pots used for strains 1000 and 1001 were replanted to the three bean varieties on January 18, 1941, and dug on March 4, 1941, when bean pods were just developing (Table 5, column 1). Strain 1000 showed a root-rot index for infection of 33 on California-grown red kidney and 67 on Yellow Eye and Jumbo Marrow or an average index of 56. Strain 1001 produced only a slight infection on all varieties in the order of an index 10, 10, and 37, with an average of 19, which was evidenced by streaking and flecking. This test would indicate again that strain 1000 could attack some beans slightly to moderately, and that strain 1001, while it did produce a slight streaking and flecking on beans, did not show any degree of pathogenicity. The foot-rot lesions of bean plants

TABLE 5.—*Root-rot index of bean varieties and wilt-resistant Perfection peas grown in soil infested with strains of Fusarium from peas and beans*

		1	2	3	4	5	6
Strain	Source	Average 3 varieties,[a] Jan. 11 & 18, Feb. 10, 1941; cornmeal culture	Average 3 varieties,[a] Feb. 18, 1941; soil replanted	Jumbo Marrow, Apr. 30, 1942; cornmeal culture	Jumbo Marrow, July 7, 1942; soil replanted	Idaho Refugee, Jan. 24, 1944; cornmeal culture	Wilt-res. Perfection, Dec. 9, 1943; cornmeal culture
1000	Bean	56[b]	51[b]	0	0	49[b]	100
1001	Pea	19[b]	33[b]	5[b]	4[b]	.....	.....
1002	Pea	24[b]	.....	3[b]	4[b]	.....	.....
1003	Pea	0	0	.....	.....	.....	.....
1004	Pea	19[b]	.....	.....	.....	.....	.....
1005	Pea	12[b]	.....	.....	.....	.....	.....
1032	Bean	.....	.....	.....	.....	56	4[b]
1033	Bean	.....	.....	.....	.....	100	29[b]
1034	Bean	.....	.....	.....	.....	100	32[b]
1035	Bean	.....	.....	.....	.....	18	3[b]
1036	Bean	.....	.....	.....	.....	49	14[b]
1037	Bean	.....	.....	.....	.....	4	0
1038	Bean	.....	.....	.....	.....	100	39[b]
1039	Bean	.....	.....	.....	.....	40	.....
1040	Bean	.....	.....	.....	.....	4	10[b]
Check		0	0	0	0	0	0

[a] California-grown red kidney, Yellow Eye, and Jumbo Marrow beans.
[b] Streaks or flecks on foot or taproot.

slightly infected with strains 1000, 1001, and 1002 in the January 11 and 18 tests were cut out and dried for use as inoculum in pea infection tests reported later under soil inoculations with infected bean roots.

On February 10, 1941, Fusarium strains 1003, 1004, and 1005 were tested on the three bean varieties planted in the infested soil from the original pea test. The plants with bean pods just developing were measured and dug for observation on April 10, 1941 (Table 5, column 1). Strain 1003 showed no sign of ability to attack the bean roots and strains 1004 and 1005 produced very slight infection evidenced by streaks or flecks. No stunting was noted, using the test with strain 1003 as representative of a check.

The important strains for further tests on beans were 1000, 1001, and 1003, the latter apparently a saprophyte, or a mild parasite on peas, to serve as a check in addition to the conventional controls. It was decided to plant each of three pots in the 1000, 1001, and 1003 series and the check of the original pot experiment, planted December 20, 1940 (Table 3), with 10 seeds each of California-grown red kidney, Yellow Eye, and Jumbo Marrow beans. Planting was done on February 18, 1941, and the plants were dug on April 10, 1941, at which time pods were developing. According to data in table 5, column 2, strain 1000 again was able to attack beans in a mild to moderate form, the root-rot index averaging 51; and strain 1001 caused a slight flecking on the taproot of all varieties, an average disease

index being 33. Strain 1003 caused no infection and all control plants were free from infection. Plant measurements showed none to possibly a slight stunting of plants growing in soil infested with strains 1000 and 1001, but no stunting of the plants grown in soil infested with strain 1003. Reisolations of the strains from diseased plants were positive.

In addition to the above test, three pots of newly sterilized soil inoculated with cornmeal-sand cultures of strains 1000, 1001, and 1002 along with a check were planted on April 30, 1942, with 10 seeds of Jumbo Marrow beans in each, and examined on July 1, 1942 (Table 5, column 3). No infection took place with strain 1000 and only a very slight streaking and flecking was produced by strains 1001 and 1002. The controls were free from any discoloration. These pots were replanted with Jumbo Marrow beans on July 7, 1942, and examined for infection on September 1, 1942. The results (Table 5, column 4) were the same as in the first test. Only very slight infection was produced on Jumbo Marrow beans by pea strains 1001 and 1002, evidenced by flecking or, at most, slight streaking. Reisolations were positive.

Inoculations with Bean Fusarium Strains

Because of these results with pea Fusarium strains, it was decided to isolate strains of fusaria from foot-rotted and root-rotted bean plants and test these strains for bean pathogenicity and for ability to go to peas. Table 1 gives the history of strains 1032 through 1040 isolated from beans. A review of this list shows that fusaria strains were isolated from mature plants some of which were apparently normal in growth while others had been stunted. It was expected that saprophytic fusaria closely related to the pathogenic forms would be isolated. Because, as indicated under morphology, the size of spores and other spore characters do not always suffice to discriminate between a pathogen and a saprophyte, inoculation tests for pathogenicity were conducted with all strains.

Bean inoculations in soil with cornmeal-sand inoculum. On January 24, 1944, tests with the various bean strains were run in pots of sterilized soil inoculated with cornmeal-sand cultures. Six Idaho Refugee bean seeds were planted per pot in triplicate tests. The pea strain 1000 isolated from bean and the bean strains 1032 to 1040 inclusive were used for inoculations. The results of these bean tests are in table 5, column 5. Disease records were taken 35 days after planting, February 28, 1944, on taproot and fibrous roots. Strains 1033, 1034, and 1038 caused severe rot to taproot and fibrous roots of beans. Strain 1000, the pea pathogen isolated from a diseased bean, caused only slight to moderate infection, as was true with strains 1032, 1036, and 1039. Only very slight discoloration was noted on roots growing in the pots infested with strains 1035, 1037, and 1040. The two latter strains produced no rot of the fibrous roots and very slight discoloration of the taproot. This test showed again that strain 1000 was not a true bean pathogen but could slightly infect beans,

and that strains 1033, 1034, and 1038, isolated from foot-rotted and root-rotted diseased beans, were virulent bean pathogens. Check plants were all healthy. The pathogens were reisolated from all infected plants.

Pea inoculations in soil with cornmeal-sand inoculum. The inoculations with bean Fusarium strains on Perfection peas started on December 9, 1943, are in table 5, column 6. The surface of all seeds was disinfected in a 0.5 per cent hypochlorite solution before planting. Data on infection were taken on January 18, 1944, when pods were developing. The test indicated that none of the strains 1032 through 1040 had any real ability to attack peas. Strains 1032, 1033, 1034, 1035, 1036, and 1038 produced very slight to moderate infection, evidenced by flecking and possibly some reduction in growth of peas. Strain 1000 again severely attacked peas, with an index of 100, and caused severe stunting. Strains 1037 and 1040 produced very slight infection or none.

TABLE 6.—*Root-rot index of Idaho Refugee beans and Thomas Laxton peas grown in soil or in sand infested with spore suspensions of strains of Fusarium from peas and beans*

Strain	Source	Beans				Peas			
		1	2	3	4	5	6	7	8
		Mar. 4, 1944 Soil	Apr. 6, 1944 Sand	May 26, 1944 Sand	Jan. 18, 1949 Sand	Mar. 4, 1944 Soil	Apr. 6, 1944 Sand	May 26, 1944 Sand	Jan. 18, 1949 Sand
1000	Bean	22[a]	23[a]	13	0	88	100	100[b]	40
1001	Pea	—	—	2	—	—	—	100[b]	—
1002	Pea	—	—	3	6	—	—	100[b]	99
1003	Pea	—	—	0	0	—	—	3[a]	31
1034	Bean	100	—	—	—	2[a]	—	—	—
1038	Bean	100	100	100	85	34[a]	23[a]	3[a]	3
1040	Bean	—	—	—	0	—	—	—	0
Check		0	0	0	0	0	0	0	0

[a] Streaks or flecks on foot or taproot.
[b] Secondary roots moderately browned.

Bean and pea inoculations in soil with standard liquid spore suspensions. In order to eliminate the possibility of organic decomposition products from the cornmeal-sand cultures influencing the results in soil inoculation tests, new bean and pea tests with strains 1000, 1034, and 1038 were run using a liquid inoculum of 50 cc. of a standard spore and hyphal suspension from an aerated liquid medium (4). These tests were run in triplicate pots of soil for each strain. Each 8-in. pot was planted with 20 seeds. The planting with Idaho Refugee beans and Thomas Laxton peas was made on March 4, 1944, and data were taken on March 24, 1944.

On beans, as indicated in table 6, column 1, strain 1000 produced only a slight flecking on the taproot, with an index of 22. The secondary roots were healthy. Strains 1034 and 1038, on the other hand, produced severe

disease with an index each of 100 on the taproot and secondary roots, and stunting of the plants. On Thomas Laxton peas (Table 6, column 5), however, strain 1000 produced severe disease, with an index of 88 on the stems and 47 on the roots, and stunted the plants; and strains 1034 and 1038 produced only a slight flecking of the foot with little to no infection of the roots and no stunting. Control plants were all healthy. Reisolations from infested plants were all positive.

Pea and bean inoculations in sand cultures with standard liquid spore suspensions. Comparative tests run with strains 1000 and 1038 in sand cultures placed in a so-called "climate chamber" with fluorescent lights and held at a temperature between 71.5° and 75° F. were started on April 6, 1944, and data were taken on April 25, 1944. The tests were run in duplicate with 12 seeds planted per crock. Inoculation of the crock sand cultures was made with a standard spore suspension. According to data in table 6, column 6, on Thomas Laxton peas, strain 1000 produced severe disease with an index rating of 100, but strain 1038 produced only flecking on stems with a disease rating of 23. The control plants were healthy. On Idaho Refugee beans (Table 6, column 2), strain 1000 produced only slight flecking with a disease rating of 23, whereas strain 1038 produced severe disease with a rating of 100. The controls were healthy. Reisolations were positive.

To round out the tests further with the virulent pea (*F. solani* v. *martii* f. *2*) and bean (*F. solani* v. *martii* f. *3*) strains, another set of sterilized sand nutrient culture tests was run in the greenhouse. Three 1-gal. crocks were used for each inoculation treatment. On May 12, 1944, 20 hypochlorite surface-disinfected seeds were planted per crock. On May 26, 1944, the surface of the sand was inoculated with 50 cc. of a standard spore and hyphal suspension from an aerated liquid medium. Data for all tests were taken on June 15, 1944, when the pea plants were starting to form pods and the bean plants were in pre-bloom stage. Fusarium strains 1001, 1002, and 1003 isolated from peas and strains 1000 and 1038 isolated from beans were used in the tests. The results of the May 26, 1944, tests on Idaho Refugee beans and Thomas Laxton peas are summarized in table 6, columns 3 and 7. Strain 1000 produced only slight flecking with a rating of 13 on Idaho Refugee beans, but severe disease with a rating of 100 on Thomas Laxton peas, indicating that it was a virulent pea parasite and not capable of producing severe disease on beans (Fig. 4, C). Strain 1001 was capable of producing only occasional flecking with a rating of 2 on beans, and severe disease with a rating of 100 on peas. Strain 1002 fell in the same category as strain 1001. Strain 1003 proved to be a saprophyte or at most a weak parasite on both beans and peas. Very slight flecking, with a rating of 3, was observed on peas. Strain 1038 proved to be a virulent bean parasite, producing severe disease with a rating of 100 on beans (Table 6, column 3) (Fig. 4, D), and only very slight

flecking on peas with a rating of 3 (Table 6, column 7). The control plants in the pea and bean tests were healthy. On peas, severe stunting of plants was produced by attacks of strains 1000, 1001, and 1002 with 34 in. as the average length of check plants as opposed to 23, 24, and 28 in., respectively, for plants growing in the infested soil. The bean strain 1038 and the pea strain 1003 apparently produced some stunting on peas with measurements of 29 and 27 in., respectively. On beans, the pea strains 1000, 1001, 1002, and 1003 caused no stunting. The bean pathogen 1038 produced severe stunting on Idaho Refugee beans, with checks measuring 9 in. and infected plants 7 in.

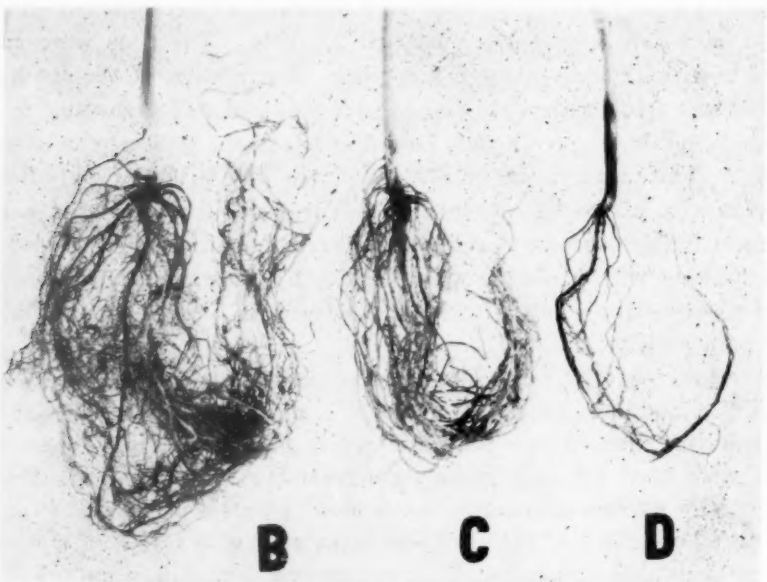

FIG. 4. Infection tests in sand nutrient cultures with pea and bean Fusarium strains on Idaho Refugee beans. Sand nutrient culture tests inoculated with standard liquid spore suspensions on May 26, 1944. Data were taken on June 15, 1944, 20 days after inoculation, when the beans were in pre-bloom stage. B. Check plant had no evidence of infection. C. The pea foot-rot and root-rot strain 1000 which was isolated from a diseased California red kidney bean caused flecking of the foot and slight root-rot. D. Severe foot-rot and root-rot produced by the virulent bean parasite, strain 1038.

A final check test was started on January 7, 1949, or 8½ and somewhat over 5 years after the original isolation of the organisms, with selected pea and bean Fusarium strains, to check again on the behavior of these strains in sand nutrient culture tests. Strain 1000, isolated from bean but according to former tests a pea parasite, along with strains 1002 and 1003 isolated from peas, were run in comparison with the virulent bean strain 1038 and the saprophytic bean strain 1040. The tests were set up in 3-gal. crocks with duplicate crocks for each strain. The crocks, sand, and all glassware were sterilized. All seeds were surface-sterilized in a 0.5 per cent hypochlorite solution for 15 min. before planting. One half of each crock was planted with 10 seeds of Thomas Laxton peas and the other half with 10 seeds of Idaho Refugee beans. The seeds were planted on

January 7, 1949, and the sand cultures with the growing plants were inoculated on January 18, 1949. The plants were dug on January 31, 1949, just 13 days after inoculation. One liter of a spore inoculum, with a concentration of approximately 170,000 spores per cc., was used per crock. At the time of inoculation the beans had fully developed primary leaves and the peas had five expanded nodes. The sand and roots of plants were automatically flushed with a nutrient solution twice a day. Data are presented in table 6, columns 4 and 8.

FIG. 5. Bean and pea inoculations with standard liquid spore suspensions in sand culture. A. Severe wilting and death of Thomas Laxton pea plants 10 days after inoculation with pea strain 1002. Idaho Refugee beans in same pot showed no signs of disease. B. Wilting and death of leaves of Idaho Refugee bean plants 9 days after inoculation with bean strain 1038. Plants severely stunted. Peas in same pot showed no signs of disease.

Nutrient sand culture tests of January 18, 1949. (Photographed January 27, 1949.)

Three days after inoculation, flecks in the pea foot just at the sand surface were noted on peas growing in the pots inoculated with strain 1002. On January 23, the peas in these crocks started to flag. On January 27, brownish lesions appeared on the foot of all plants, along with wilting of the lower leaves. On January 28, all peas were severely wilted and two plants were dead (Fig. 5, A). The bean plants in these pots appeared to be normal. The plants were dug on January 31, 13 days after inoculation, and all pea plants had severe blackening of the foot and taproot extending above and below the seed attachment (Fig. 6, A). The seeds were all rotted. The peas had a root-rot index of 99 (Table 6, column 8). Most bean stems and roots were healthy, but a number of plants had minute flecks to larger streaks of infection (Fig. 6, A). The average root-rot index on beans was calculated to be 6 (Table 6, column 4).

Strain 1000 isolated from a diseased bean, but which actually in former tests proved to be a pea parasite, again performed as in previous tests. Flecking was noted on the foot of pea plants on January 27, and by January 28 slight flagging of two plants was evident. No other plants wilted. When dug on January 31, the foot regions of all plants were badly diseased

(Fig. 6, B). The average root-rot index was 40 (Table 6, column 8) on plants dug 13 days after inoculation. About one-half of the seeds were rotted. This behavior was similar to that in the original inoculations of December 20, 1940, showing a distinct pea parasite but one not so virulent as strain 1002. The beans growing in the same pots all had some very minute flecking, indicating penetration of the fungus, but showed no severe infection when dug 13 days after inoculation (Fig. 6, B).

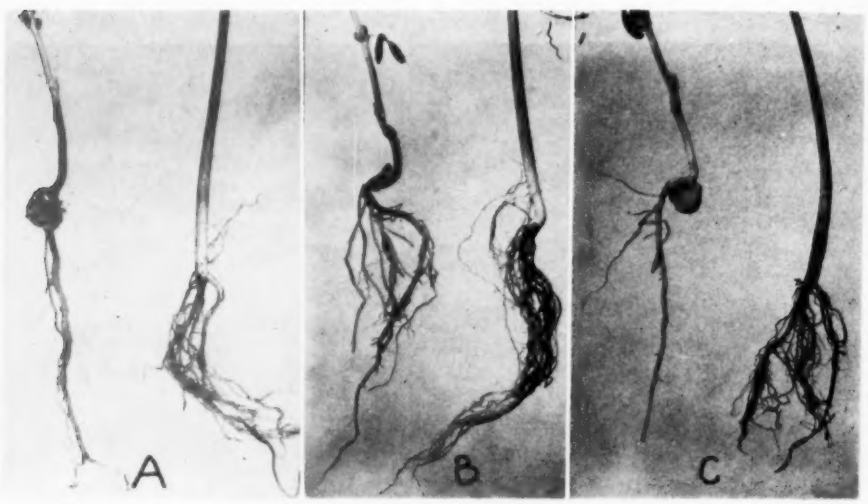

FIG. 6. Bean and pea inoculations with standard liquid spore suspensions in sand cultures. A. Virulent pea root-rot strain 1002, 13 days after inoculation, produced severe foot-rot on Thomas Laxton peas and slight flecking to streaking on Idaho Refugee beans. On some beans the infection was somewhat more severe. B. Pea root-rot strain 1000 isolated from a diseased California red kidney bean proved to be a virulent pea root-rot strain which did not always attack beans. C. Virulent bean root-rot strain 1038, 13 days after inoculation, produced severe foot-rot and root-rot of Idaho Refugee bean and some minute flecking of the foot of Thomas Laxton peas.

Nutrient sand culture tests of January 18, 1949, 13 days after inoculation. (Photographed January 31, 1949.)

Strain 1003 in this test showed a slight degree of parasitism on peas with a root-rot index of 31 (Table 6, column 8). None of the peas had wilted. The pea seeds were badly rotted on all plants. In the previous tests, the strain behaved more nearly like a saprophyte with only occasionally a very slight attack on peas. The organism showed no real infection of beans; only a few flecks on bean stems were produced.

Strain 1038 isolated from bean lived up to its reputation as a virulent bean parasite, with a disease index rating of 85 (Table 6, column 4) (Fig. 6, C). By January 17, a few leaves on a number of plants were wilted and brown, and later fell (Fig. 5, B). The foot portions of all plants were reddish-brown and badly diseased and had started to crack. Some of the secondary roots were slightly infected. All plants were badly stunted. On peas, only slight flecking was produced on the lower stems (Fig. 6, C). Only two seeds were decayed on the pea plants.

Strain 1040 isolated from beans showed no real sign of infection on

either peas or beans when dug on January 31 (Table 6, columns 4 and 8) The seeds of the peas were only slightly decayed. Very minute flecking was produced on the stems of both peas and beans.

Reisolations from all diseased plants were positive for each strain that caused infection.

Soil Inoculations with Bean Roots Infected by Pea Strains

Diseased bean roots from the inoculation tests of January 11 and 18, 1941, in which pea strains 1000, 1001, and 1002 showed some ability to attack bean roots, were collected and dried for the purpose of infesting sterilized soil for pea tests. The affected beans were dug and diseased portions cut out and dried on March 4, 1941. Two pots of sterilized soil in a series were inoculated with diseased portions of bean roots of plants infected by pea strains 1000, 1001, and 1002. On March 7, 1941, each pot was planted with 10 Thomas Laxton pea seeds. When the peas were in blossom on April 10, 1941, they were dug for observation. All three strains produced severe disease with root-rot indexes 80, 90, and 95, respectively. The control plants in pots of sterilized soil planted with peas showed no sign of disease. The combined greenhouse tests indicated that pea strains 1000, 1001, and 1002 could mildly attack beans and that diseased parts of the bean plants when introduced into sterile soil could infest soil and provide inoculum for infection of peas.

SUMMARY AND CONCLUSIONS

The Fusarium type for the pea root-rot organism, *F. solani* v. *martii* f. *2*, has shorter and narrower spores, measuring 38×4.8 μ, than the type for the bean root-rot form, *F. solani* v. *martii* f. *3*, which has average spore measurements of 44×5.5 μ (15, 16). The virulent pea strains used in these tests were slightly shorter and broader than the type. The moderately virulent strains agreed more closely with the type. The virulent bean strains agreed very closely with the type form. The mildly parasitic strains measured somewhat smaller. The saprophytic or at most slightly parasitic strains used in each group as checks, typified by pea strain 1003 and bean strain 1040, were off-types. The pea strain 1003 was consistently shorter and broader, whereas the bean strain 1040 was shorter and narrower.

The various pea and bean strains in culture varied somewhat in growth characters and color production. The latter characteristic, however, is not always distinctive for Fusarium in the Martiella section. In cases of wide difference, however, it may be an aid in identification. Production of dense masses of pionnotes is characteristic for both pea and bean forms. Pathogenic pea and bean strains normally produced a blue-green coloration in the pionnotes mass. At times, however, only green and cream-buff pionnotes masses may be produced.

The morphological and cultural studies showed that by these means it was possible to pick out some closely allied saprophytic strains as verified by inoculation studies. In general, however, inoculation tests are really

the only safe test for establishment of the true parasitic strains of both the pea and bean organisms.

Studies by other investigators showed that the root-rot pea and bean forms of Fusarium were distinct and that one could not attack the host of another (3, 11). In those studies, one strain of each was used for inoculations. Because of differences in virulence of strains shown in preliminary inoculation experiments (8), along with morphological and cultural differences, and because sectoring took place in some of the single-spore pea root-rot strains, it was thought that strains might exist that could go from pea to bean, or the reverse. This assumption found further support in that strain 1000 isolated from a diseased bean proved to be a virulent pea parasite. The fact that the pea form was isolated from a growing diseased bean plant showed that it could exist on the foot or roots of beans but did not prove that it was a virulent parasite on beans.

The studies showed the presence of pea and bean strains within the types that varied in degree of virulence to their respective hosts. For example, strain 1002, from pea, isolated June 14, 1940, wilted and killed pea plants much faster than any other pea strain isolated. This strain has retained its extreme virulence for somewhat over 8 years, as indicated in the latest test on January 7, 1949.

All tests from 1937 to date proved that there is a distinct pea parasite and a distinct bean parasite. In no case was severe disease consistently produced by the pea pathogens on beans or the reverse. The tests did show that some pea strains could attack beans in a minor way and that to a less degree some of the bean strains could slightly infect peas. Those pea strains which attacked beans and the bean strains which attacked peas produced more infection when the peas or beans were allowed to remain in infested soil or sand up to the time of blooming or pod formation. In other words, the hosts were more susceptible at flowering and fruiting time.

The investigations further showed that pea strains which slightly infected beans could live on bean roots at least as saprophytes and then were capable of infesting soil and reinfecting peas. This fact was further substantiated by strain 1000, isolated from diseased roots of a field-grown California red kidney bean, which in inoculation tests proved to be a virulent pea root-rot strain capable of only slightly attacking beans. The significance of this test would have to be amplified for further evidence of carry-over of pea strains on beans and the possible effect on control. It was not shown how prevalent this might be in the field and whether or not the same conditions might not exist with other hosts than beans. Other investigators (1, 2, 6) have shown that plants other than the host may act as carriers.

The cross inoculation tests on peas and beans were first run in pots of soil infested with cornmeal-sand inoculum. Since it was thought that the streaking and flecking obtained in the first cross inoculations might have been caused by organic decomposition products from the inoculum,

tests were run using liquid spore suspensions of the organisms in soil and lastly in sand cultures. In both instances, in cross inoculation tests, some flecking and streaking on peas and beans occurred, thereby substantiating the tests in which cornmeal-sand cultures were used as inoculum. It was concluded that the cornmeal-sand cultures, in the amounts used in pots of soil for the first tests, had no effect on the roots of the plants and that the streaking and flecking produced must have arisen from infections. Positive reisolations of the inoculum used in each instance further substantiated these findings.

The flecking and slight streaking produced on beans by pea Fusarium strains and on peas by the bean Fusarium strains would indicate definite resistance of each host to Fusarium strains isolated from opposite hosts. Because of lack of infection on individuals in some tests, it appeared that individual pea and bean plants of one variety differed in susceptibility. None of the varieties used was genetically pure.

AGRICULTURAL EXPERIMENT STATION
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LITERATURE CITED

1. ARMSTRONG, G. M., and JOANNE K. ARMSTRONG. Nonsusceptible hosts as carriers of wilt Fusaria. Phytopath. **38**: 808–826. 1948.
2. ————, and ————. The Fusarium wilt of cowpeas and soybeans. (Abstr.) Phytopath. **39**: 1–2. 1949.
3. BURKHOLDER, WALTER H. The dry root-rot of the bean. Cornell Univer. Agr. Exp. Sta. Memoir **26**: 999–1033. 1919.
4. GRIES, GEORGE A. The effect of plant-decomposition products on root diseases. (Abstr.) Phytopath. **33**: 1111–1112. 1943.
5. HARTER, L. L. Influence of light on the length of the conidia in certain species of Fusarium. Amer. Jour. Bot. **26**: 234–243. 1939.
6. HENDRIX, J. W., E. P. DUCHARME, and HARRY MURAKISHI. Pathogenicity of single spore isolates of the fusarium-wilt organisms of muskmelon and watermelon. (Abstr.) Phytopath. **38**: 13. 1948.
7. PRASAD, N. Variability of the cucurbit root-rot fungus, Fusarium (Hypomyces) solani f. cucurbitae. Phytopath. **39**: 133–138. 1949.
8. REINKING, O. A. Distribution and relative importance of various fungi associated with pea root-rot in commercial pea-growing areas in New York. N. Y. State (Geneva) Agr. Exp. Tech. Bul. 264. 1942.
9. ————, and H. W. WOLLENWEBER. Tropical Fusaria. The Philippine Jour. of Sci. **32**: 103–253. 1927.
10. SNYDER, WILLIAM C. Notes on Fusaria of the section Martiella. Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten. Part II. **91**: 163–184. 1934.
11. ————. St. John's disease of pea in Europe. Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten. Part II: **91**: 449–459. 1935.
12. ————, and H. N. HANSEN. The importance of variation in the taxonomy of fungi. Proc. Sixth Pacific Science Congress **4**: 749–752. 1939.
13. WELLMAN, FREDERICK L., and DOROTHY J. BLAISDELL. Differences in growth characters and pathogenicity of Fusarium wilt isolations tested on three tomato varieties. U. S. Dept. Agr. Tech. Bul. 705. 1940.
14. ————, and ————. Pathogenic and cultural variation among single-spore isolates from strains of the tomato-wilt Fusarium. Phytopath. **31**: 103–120. 1941.
15. WOLLENWEBER, H. W. Fusarium-Monographie. II. Fungi parasitici et saprophytici. Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten. Part II. **106**: 104–202. 1943.
16. ————, and O. A. REINKING. Die Fusarien. 355 pp. Paul Parey, Berlin. 1935.
17. ————, C. D. SHERBAKOFF, O. A. REINKING, HELEN JOHANN, and ALICE A. BAILEY. Fundamentals for taxonomic studies of Fusaria. Jour. Agr. Res. [U.S.] **30**: 833–843. 1925.

THE RELATION OF BEAN VIRUS 2 TO PEA MOSAIC IN WISCONSIN[1]

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INTRODUCTION

The causal agent of yellow bean mosaic, bean virus 2, was found on peas (*Pisum sativum* L.) for the first time in Wisconsin near Madison in 1946. Because this virus, which caused distinct leaf mottle on pea, was found to be widespread on that host and was somewhat different from other strains of bean virus 2, it was chosen for detailed study.

Several investigators have described mosaic viruses of peas in Wisconsin (1, 11, 13), but no reports were given as to the prevalence of viruses concerned. Observations made during the growing seasons of 1946 and 1947 indicated that the mosaic disease of pea incited by bean virus 2 was widespread in southern Wisconsin. To obtain a more complete state-wide picture, samples of mosaic-diseased pea plants were collected in 1948 from fields in 12 Wisconsin counties where canning pea culture is important. Results of selective host inoculations from these samples indicated that pea mosaic caused by bean virus 2 was the most prevalent virus disease of peas in Wisconsin, having been present in 14 separate fields. Enation pea mosaic (11) was encountered only once, the pea stunt virus (5) was recovered 11 times, and the Wisconsin pea streak virus seven times. A strain of cucumber virus 1 causing mosaic and death of pea was also recovered on two occasions.

To substantiate the identification of the virus isolated in 1946 as a strain of bean virus 2, this paper will report the detailed studies conducted with it; evidence that it is a new strain will be presented. It will be referred to as isolate 1 of bean virus 2. Certain sections of this report will give a comparison of isolate 1 with bean virus 2 strains recovered from diseased peas collected in Dane County, Green Lake County, and Winnebago County, Wisconsin. They are referred to, respectively, as isolate 2, isolate 3, and isolate 4. Preliminary reports have already been made (3, 4).

METHODS AND MATERIALS

Throughout most of the greenhouse studies the plants were grown in compost soil in 6-in. earthenware pots at temperatures of 20° to 24° C. The Perfected Wales variety of peas was used as a culture plant and for

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most of the experiments with that host because it is very susceptible to the mosaic virus.

The rubbing method of inoculation, using a cheesecloth pad, was found most successful. Carborundum powder, sprinkled lightly on the leaves, greatly aided infection. The second and third leaves of pea were inoculated when they became fully expanded. Other hosts were inoculated similarly when they were young.

Physical properties were determined in a manner similar to that described by Johnson and Grant (7) except that aging tests were conducted at a temperature of about 22° C. (72° F.) instead of 80°–90° F.

Suitable controls were provided for all experiments conducted.

EXPERIMENTAL RESULTS

Symptoms

The most conspicuous symptom on pea plants in the field infected by isolate 1 was a distinct mottle of the foliage (Fig. 1) caused by chlorosis of the tissue between the veins. Patches of normal green tissue were scattered irregularly around the leaf and stipule surfaces. These green areas were of various sizes and shapes, but most of them covered an area of 2 to 4 square millimeters. No veinclearing was observed. The upper leaves and stipules were slightly wrinkled, and abnormal axillary bud growth was present in most cases. Diseased plants were somewhat stunted if infected when young. The general color of the plant was almost as deep a green as healthy plants. No symptoms were apparent on the pods or stems.

Symptoms on Perfected Wales peas in the greenhouse were less striking than in the field. Often the first symptom was a chlorosis and wilting of inoculated leaves and of their adjacent stipules. Inoculated leaves remained attached. About 10 days after inoculation a very slight clearing of the larger veins was sometimes apparent in the apical leaflets. Small, faint, chlorotic spots, producing a very faint mottle, could then be seen between the veins of the youngest leaves. In many cases this was the first foliage symptom, veinclearing being absent. Ordinarily a skip of two sets of leaves was made from inoculated leaves to the first leaves showing the systemic mottle. After 12 to 16 days the characteristic mosaic (Fig. 2, B, C), similar to that found in the field, had developed on the leaves and stipules. Number and size of the normal green areas varied greatly. Sometimes very few of these spots were present and they were near the base of the leaflet, leaving the apical portion devoid of such green areas. There was little, if any, plant stunt or reduction of leaf size. Occasional slight curling or wrinkling of the apical leaves and stipules was observed. A mild general chlorosis of the plant was apparent in contrast to healthy plants. Symptoms produced on other varieties of peas tested were similar to those described above. Symptoms of the other three isolates of bean virus 2 were very similar to those just described (Fig. 2).

Of the bean varieties tested, the one most severely affected was white-seeded Stringless Blue Lake. Eighteen days after inoculation the unifoliate leaves showed necrosis of the veins and pronounced necrotic streaks on the stem and leaf petioles; stunting of the plant was very severe (Fig. 3),

FIG. 1. Pea plant showing symptoms of the isolate 1 of bean virus 2 as seen in the field in 1946. Note leaf mottle, axillary bud growth, and slight wrinkling of apical foliage.

and only two sets of trifoliate leaves had developed as compared to five for healthy plants. The trifoliate leaves of infected plants were severely dwarfed, and apical malformation and downward curling were very conspicuous. Infected leaves also showed veinal and interveinal spot necrosis

in addition to severe roughening of the leaf surface. Portions of these severely dwarfed leaves died from the tip backward; sometimes whole leaves were killed.

Diseased Michelite bean plants were dwarfed only slightly, but a striking mottle resulted from irregular veinal and interveinal chlorotic areas on the leaves. In some cases the number and size of these yellowed spots increased until most of the leaf surface was chlorotic, leaving only a few large scattered patches of normal green tissue. Infected leaves were wrinkled and were reduced in size. Slight necrosis of the stem and petiole was also noted but no top necrosis was observed. Similar symptoms were produced on Great Northern U. of I. No. 1 (Fig. 4, B).

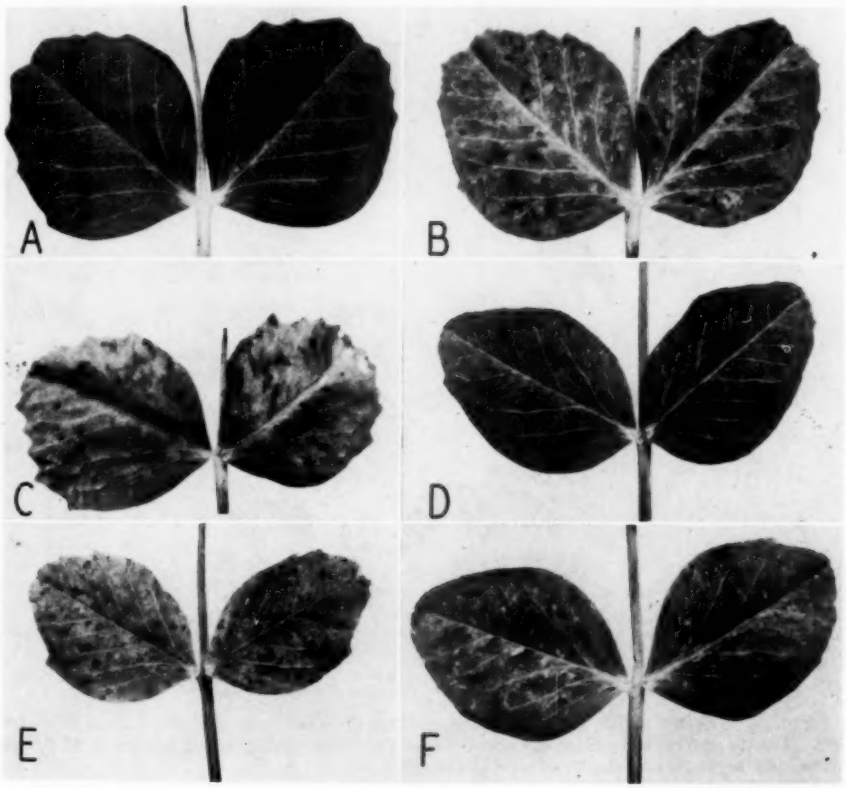

FIG. 2. Leaf mottle of Perfected Wales pea in the greenhouse, incited by four isolates of bean virus 2 found on pea in Wisconsin. A, healthy; B and C, isolate 1; D, isolate 2; E, isolate 3; F, isolate 4.

Symptoms on Idaho Refugee bean were manifested as a moderate plant stunt, unifoliate leaf epinasty, reduction in trifoliate leaf size, conspicuous leaf mottle, and slight downward curling of these leaves. The diseased leaves were slightly roughened. The yellowed areas of the mottle were often near the veins and had rather definite but irregular margins (Fig. 4, C).

The only symptom noted on diseased Red Kidney beans was a mottle of the leaves which was characterized by large areas of normal green tissue of definite margin and surrounded by an even and general chlorosis of the leaf (Fig. 4, D).

The symptoms of isolates 2, 3, and 4 were studied on Idaho Refugee bean and were found to be very similar to those of isolate 1 on that host. However, isolate 1 caused the most distinct leaf mottle and the tendency for the chlorotic areas to be adjacent to the veins was most apparent on bean

FIG. 3. The effect of isolate 1 of bean virus 2 on white-seeded Stringless Blue Lake bean. Plants on right are diseased and typically show severe stunting, epinasty of primary leaves, and necrosis and death of apical foliage.

leaves infected with this virus isolate. Smaller chlorotic areas were typical leaf symptoms of isolate 4. Symptoms produced by isolate 3 were very similar to those of isolate 1. Leaves infected with isolate 2 showed slightly larger chlorotic areas than were typical of the others.

No symptoms were observed on the pods of any bean varieties studied.

Local necrosis developed on soybean (*Soja max* (L.) Piper) leaves inoculated with isolate 1, but three attempts to recover the virus from these leaves were unsuccessful. The infection was systemic, however, because the virus was recovered from chlorotic upper leaves.

Isolate 1 produced a variety of symptoms on the different clovers tested. On red clover (*Trifolium pratense* L.) and crimson clover (*T. incarnatum* L.) the first symptom was veinclearing followed by a mottle caused by interveinal chlorotic areas (Fig. 5, B, D). In the case of red clover these yellowed areas became very pronounced. Wrinkling and dwarfing of the

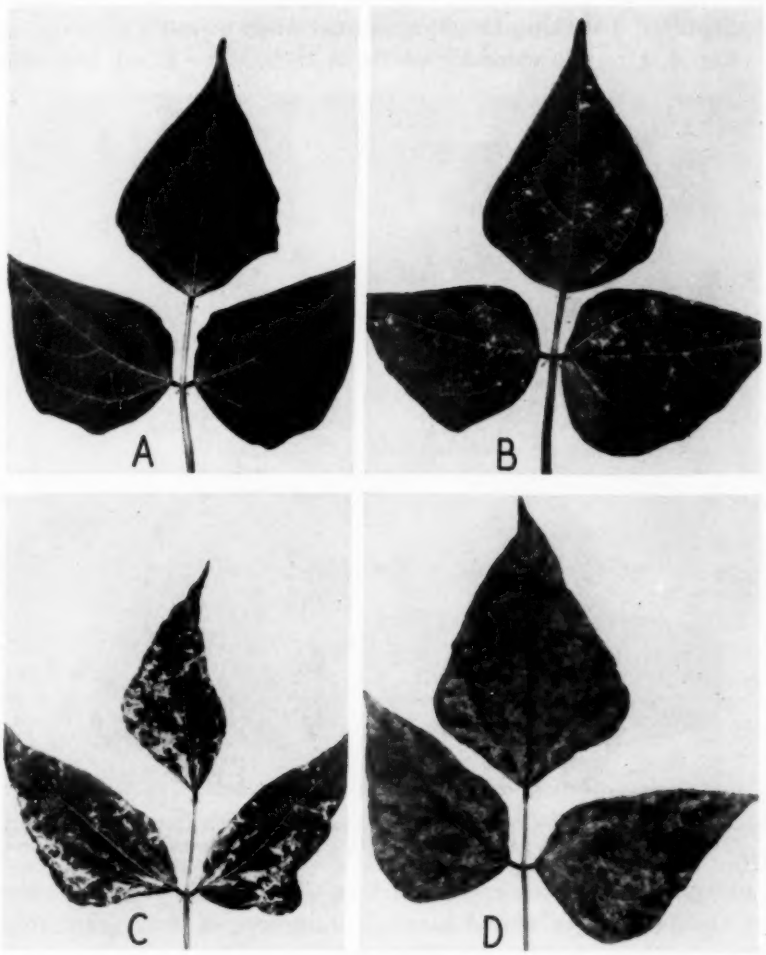

FIG. 4. Leaf symptoms of bean virus 2 isolate 1 on beans. A, healthy Great Northern U. of I. No. 1; B, diseased Great Northern U. of I. No. 1; C, diseased Idaho Refugee; D, diseased Red Kidney.

leaves were moderate on both these hosts. Diseased crimson clover plants were severely dwarfed. When alsike clover (*T. hybridum* L.) was infected, veinclearing was absent, but a faint mottle was noted. This symptom was the result of small chlorotic areas (Fig. 5, C) developing between the veins. These spots were never yellow, but rather a light green, and were peculiar in that their centers often contained a small amount of normal green tissue.

Slight wrinkling and dwarfing of diseased alsike clover leaves were also observed.

Symptoms of isolate 1 on the sweet clovers were rather distinct. Diseased white sweet clover (*Melilotus alba* Desr.) leaves showed veinclearing, and later mosaic mottling brought about by chlorotic areas of various sizes between the veins (Fig. 6, B). Yellow sweet clover (*M. officinalis* (L.) Lam.) displayed a striking mosaic symptom when infected with the mosaic virus (Fig. 6, C). No chlorotic spots, as such, were noted, but yellowed

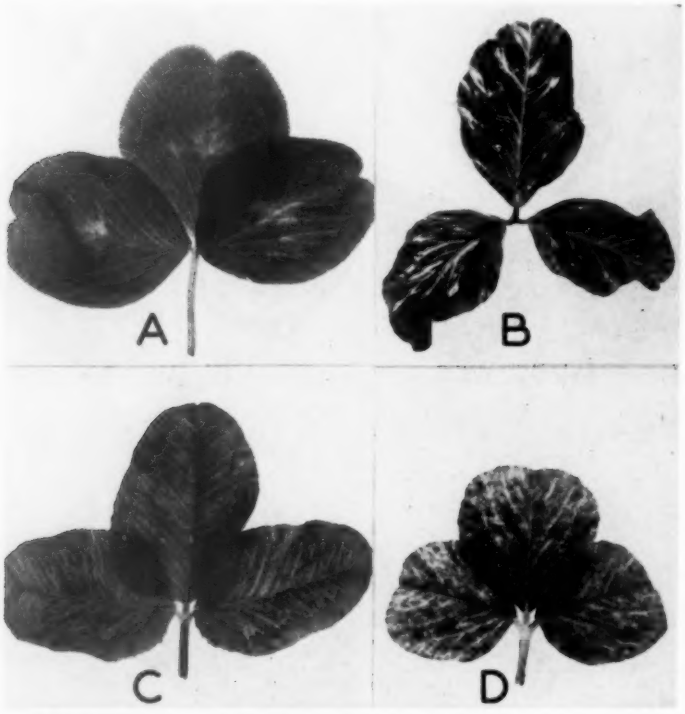

FIG. 5. Leaf symptoms of isolate 1 of bean virus 2 on clovers. A, healthy red clover; B, diseased red clover; C, diseased alsike clover; D, diseased crimson clover.

areas unbounded by veins and of various sizes and shapes were apparent. Almost always such discolored places had an area of dark green tissue on one border and sometimes these dark green splotches were surrounded by the chlorosis. The yellowed areas on this host had no distinct margins except along the dark green spots.

The other three bean virus 2 isolates caused symptoms on the above leguminous hosts which were very similar to those described for isolate 1. Figure 6 illustrates the symptoms of all four virus isolates on yellow sweet clover.

Temperature Relations

The effect of temperature on disease development and symptoms of the mosaic virus on peas was mainly one of varying the incubation period.

At 16° C. the typical mosaic did not develop until 48 days after inoculation; at 20° C., inoculated plants showed characteristic mosaic symptoms after 20 days; at 24° C. these symptoms developed in 15 days, and at 28° C. the incubation period was only 12 days. Infected plants did not die prematurely but continued growth and matured in a normal manner. Thus, it was evident that higher temperatures decreased the time needed for characteristic mosaic symptom development. Similar leaf symptoms were produced at the four temperatures studied.

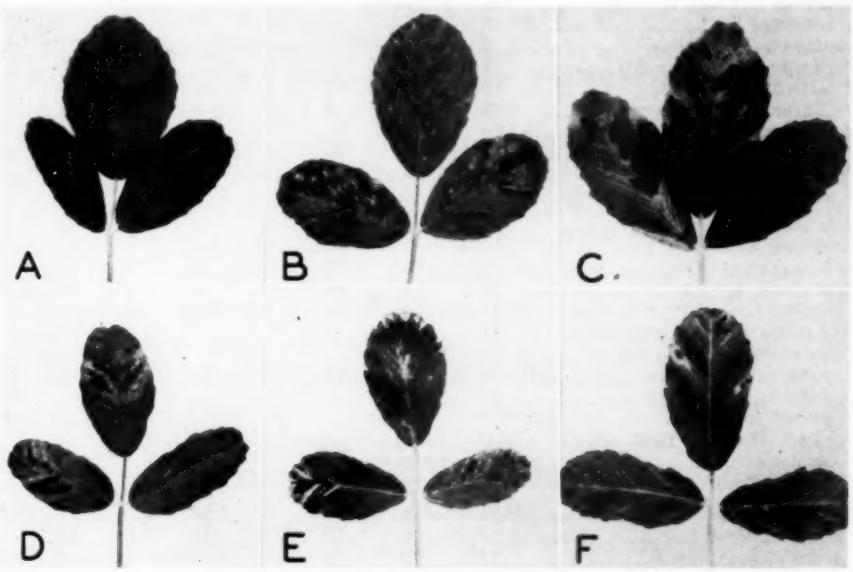

FIG. 6. Leaf symptoms of four bean virus 2 isolates from pea on sweet clover. A, healthy white sweet clover; B, isolate 1 on white sweet clover; C, isolate 1 on yellow sweet clover; D, isolate 2 on yellow sweet clover; E, isolate 4 on yellow sweet clover; F, isolate 3 on yellow sweet clover.

Host Range

Twenty-nine members of the Leguminosae, Solanaceae, and Cucurbitaceae were tested for susceptibility to isolate 1. The plants tested and the results obtained are given in table 1. This table also indicates the results obtained when some of the more common hosts were tested with the other three isolates. Inoculations from the various test plants back to healthy Perfected Wales peas were made to check recoverability of the virus. When the virus was recovered, although no symptoms were observed on the host, the word "latent" was used to show that the host was susceptible. If symptoms were absent or questionable and the virus was not recovered, the plant was considered nonsusceptible.

All four isolates of bean virus 2 from pea were limited in host range to the Leguminosae. They were not infectious to cucumber (*Cucumis sativus* L.), jimsonweed (*Datura stramonium* L.), tomato (*Lycopersicon esculentum* Mill.), and tobacco (*Nicotiana tabacum* L.). In addition, isolate 1 did

TABLE 1.—*Host range of four isolates of bean virus 2 from pea*

Host	Virus isolate 1		2		3		4	
	No. of plants		No. of plants		No. of plants		No. of plants	
	Inoculated	Diseased	Inoculated	Diseased	Inoculated	Diseased	Inoculated	Diseased
Lathyrus hirsutus L.	18	Latent[a]	....	....	....	....	....	....
L. odoratus L.	10	5	....	....	....	....	....	....
Lupinus angustifolius L.	8	Latent	....	....	....	....	....	....
Medicago orbicularis All.	7	5	....	....	....	....	....	....
M. sativa L.	13	0	14	0	15	0	16	0
Melilotus alba Desr.	26	19	4	4	4	1	4	2
M. officinalis (L.) Lam.	29	17	10	6	7	1	16	7
Phaseolus vulgaris L.	29	24	31	26	40	31	32	26
Pisum sativum L.	44	25	56	38	63	48	56	40
P. sativum var. *arvense* Pior.	10	4	....	....	....	....	....	....
Soja max (L.) Piper	20	4	14	4	14	8	14	5
Trifolium hybridum L.	25	3	16	2	15	3	12	3
T. incarnatum L.	15	10	15	12	16	9	13	9
T. pratense L.	48	34	26	1	15	4	16	4
T. repens L.	21	0	15	0	23	0	18	0
Trigonella foenum graecum L.	14	9	....	....	....	....	....	....
Vicia atropurpurea Desf.	17	Latent	....	....	....	....	....	....
V. faba L. var. *major*	9	4	14	8	11	7	16	10
V. faba L. var. *minor*	11	11	....	....	....	....	....	....
V. grandiflora Scop.	9	2	....	....	....	....	....	....
V. monantha (L.) Desf.	16	Latent	....	....	....	....	....	....
V. sativa L.	8	Latent	....	....	....	....	....	....
V. villosa Roth.	10	Latent	....	....	....	....	....	....
Vigna sinensis Endl.	19	0	16	0	6	0	13	0
Cucumis sativus L.	22	0	10	0	15	0	10	0
Datura stramonium L.	29	0	6	0	6	0	6	0
Lycopersicon esculentum Mill.	7	0	10	0	10	0	10	0
Nicotiana rustica L.	8	0	....	....	....	....	....	....
N. tabacum L.	8	0	10	0	10	0	10	0

[a] Latent = No symptoms, but virus recovered.

not infect *Nicotiana rustica* L. Nonsusceptible legumes were alfalfa (*Medicago sativa* L.), Ladino white clover (*Trifolium repens* L.), and black cowpea (*Vigna sinensis* Endl.).

The four isolates did infect and produce similar symptoms on the following leguminous hosts: white sweet clover, yellow sweet clover, red clover, bean, pea, soybean, alsike clover, crimson clover, and broad bean (*Vicia faba* L.).

No symptoms were produced by isolate 1, but the virus was recovered from rough peavine (*Lathyrus hirsutus* L.), blue lupine (*Lupinus angustifolius* L.), purple vetch (*Vicia atropurpurea* Desf.), monantha vetch (*V. monantha* (L.) Desf.), spring vetch (*V. sativa* L.), and hairy vetch (*V. villosa* Roth.). Veinclearing and mosaic symptoms were noted on five out of seven button medic plants (*Medicago orbicularis* All.), but no symptoms developed on peas when two attempts to recover the virus were made. In

addition to the hosts already named, this virus also infected the following legumes causing veinclearing and mosaic symptoms: sweet pea (*Lathyrus odoratus* L.), field pea (*Pisum sativum* var. *arvense* Poir.), fenugreek (*Trigonella foenum graecum* L.), and showy vetch (*Vicia grandiflora* Scop.). Thus it was found that 21 out of the 24 legumes tested were susceptible to isolate 1, and that the common host range of all the isolates was similar.

Pea and Bean Varietal Reactions

The reaction of 18 pea varieties and 12 bean varieties to isolate 1 was studied in the greenhouse.

Two separate trials were made with the pea varieties and similar results obtained both times. Only six of the 18 varieties were susceptible. They were Alaska, Alderman, Glacier, Perfected Wales, Profusion, and Thomas Laxton. Similar symptoms were noted, and the causal virus was recovered readily. Nonsusceptible varieties included Bonneville, Canner King, Delwiche Commando, Horal, Improved Penin, Loyalty, Merit, Pride, Resistant Famous, Wasatch, Wisconsin Early Sweet, and Wisconsin Perfection. Inoculated plants of these varieties developed no symptoms and did not yield the virus when recovery was attempted.

All 12 bean varieties tested were susceptible to the virus. They were Bountiful, Dwarf Horticultural, Great Northern U. of I. No. 1, Great Northern U. of I. No. 59, Idaho Refugee, Michelite, Pinto, Red Kidney, Sensation Refugee 1066, Stringless Blue Lake (black-seeded), Stringless Blue Lake (white-seeded), and Stringless Green Refugee. Symptoms produced varied according to variety as described above. In each case the causal virus was recovered when inoculations back to pea were made.

Physical Properties

A series of tests was made to determine the physical properties of virus isolate 1. The following properties were studied: tolerance to dilution, resistance to aging *in vitro*, and thermal inactivation. Table 2 gives the data obtained in these determinations. Results indicated that this virus was tolerant to a dilution of 1:10,000 but was noninfectious at higher dilutions. Aging for 48 hr. was sufficient to render the virus noninfective. When thermal inactivation tests were made, it was found that a temperature of 60°–64° C. was needed to completely inactivate the virus.

Transmission

When insect transmission studies were undertaken, healthy pea aphids (*Illinoia pisi* (Kalt.)) were allowed to feed on diseased plants, ordinarily to remain 2 days before removal to healthy plants. Six to 14 aphids were placed on each plant. Insects were confined to the plants by means of glass lamp chimneys. In most cases aphids were transferred by excision of the leaf on which they were feeding and removal of it to the desired location.

TABLE 2.—*Physical properties of Wisconsin isolate 1 of bean virus 2 from pea*

Type and degree of treatment	Trial 1		Trial 2		Trial 3	
	A[a]	B	A	B	A	B
Dilution:						
0	17	4	69	7	20	13
1: 10	29	7	43	11	20	14
1: 100	32	5	40	8	22	8
1: 1,000	30	2	36	3	24	1
1: 10,000	19	0	51	1	20	1
1: 100,000	....	....	43	0	22	0
1: 1,000,000	....	....	....	....	18	0
Aging:						
0 hr.	17	4	8	6	9	5
12 hr.	31	31	9	1	10	2
1 day	30	0	10	0	10	4
2 days	....	....	11	0	10	0
4 days	....	....	....	....	11	0
Heat:						
Unheated	36	6	9	5	9	2
54° C.	42	0	....	....	10	2
56° C.	36	3	11	1	10	0
58° C.	42	2	11	0	8	1
60° C.	41	2	6	0	8	0
62° C.	....	....	11	0	10	0
64° C.	40	0	10	0	9	0

[a] A = Number of plants inoculated, B = Number of plants diseased.

At the end of the feeding time on healthy plants, aphids were killed by spraying with an aphicide.

In each of the three tests very good infection was obtained, as was shown by the fact that the virus infected a total of 15 out of 15 healthy plants upon which infectious aphids had been placed. Thus it was demonstrated that this virus was very easily transmitted by the pea aphid.

An experiment was conducted at Madison in 1947 to study the natural transmission of the virus in the field. Perfected Wales peas were planted in 6-in. rows in a plot 15×35 ft., and were allowed to attain a height of about 6 in. before inoculation. Inoculation was successfully accomplished by transplanting a mosaic-diseased plant, on which 12 aphids had been feeding for 2 days, into the center of the plot and allowing the aphids to remain on the inoculum source plant. No disease symptoms were present in the plot at that time. The spread of the mosaic disease from the original source plant was noted at successive periods. Each time disease readings were taken, samples of plants considered infected were taken for check inoculation to healthy greenhouse plants to see whether or not the mosaic disease under study was present.

First symptoms of the mosaic were noted 16 days after inoculation. Plants adjacent to the diseased inoculum plant were beginning to show the typical pea mosaic at that time. Symptoms were absent in the outlying areas of the plot. Nine days later mosaic symptoms on the adjacent plants, and on others scattered around the plot, were more definite. At this time

disease counts of 100 plants each were made in four different locations of the plot, two near the inoculum source plant and two at the plot extremities. Near the transplanted inoculum plant, 31 plants were diseased out of 100 surveyed in one case; in the other, 34 plants were infected out of 100. At the northern extremity of the plot 30 plants out of 100 were diseased; 32 out of 100 were infected at the opposite end of the plot. Thirty-seven days after inoculation disease counts were again made in a manner similar to that described above. Near the center of the plot 38 and 44 plants per 100 were diseased and at the extremities 40 and 36 plants in each 100 examined were infected. A moderate pea aphid population was observed throughout the plot during the course of this study. These observations suggest that the isolate 1 virus is gradually and readily transmitted by pea aphids in the field.

In the study made to test seed transmissibility, 916 plants were grown from seeds collected from plants infected with isolate 1 in the field. No virus disease symptoms developed on any of the 916 plants. Seed was harvested from infected Red Kidney bean plants growing in the greenhouse. Two hundred six plants grown from this seed were free of any disease symptoms.

Cross Protection Tests

A distinct leaf mottle was the main symptom of the four isolates in diseased Michelite beans. Grogan and Walker (2) found, through use of cross protection tests, that the typical strain of bean virus 2 would reduce symptom severity (top necrosis) of the pod-distorting strain in that host. Similar tests were conducted with the four isolates from pea to determine whether or not they could likewise cause that lessening effect. The results obtained are summarized in table 3.

TABLE 3.—*Cross protection by four Wisconsin isolates of bean virus 2 from pea against the pod-distorting virus*

Treatment	Number of plants			
	Inoculated	Malformed	Severely stunted	Moderately stunted
Isolate 1 only	6	0	0	0
Do + pod-distorting	16	0	0	0
Isolate 2 only	7	0	0	0
Do + pod-distorting	17	0	0	0
Isolate 3 only	6	0	0	0
Do + pod-distorting	14	0	0	0
Isolate 4 only	6	0	0	0
Do + pod-distorting	18	0	0	0
Pod-distorting only	23	22	13	10

The pod-distorting strain did not cause top necrosis of Michelite bean in these tests but did produce a very marked malformation and stunting of the plant, compared to leaf mottle only, caused by the four pea isolates. Previous inoculation of plants with any of the isolates of bean virus 2 from

pea greatly reduced the severity of pod-distorting symptom expression. In no case were any of these plants severely stunted or malformed and only two or three plants were slightly stunted; whereas the pod-distorting strain alone produced 10 moderately stunted and 13 severely stunted plants. All but one of these stunted plants were also malformed. These results show clearly that all four isolates from pea are similar to typical bean virus 2 in ability to reduce severe symptom expression of the pod-distorting strain.

DISCUSSION

The mosaic virus just described is generally similar to those of the bean yellow mosaic virus group, as compiled by Weiss (12) in symptoms, host range, ability to infect many varieties of beans, nontransmissibility by bean seed, and inability to attack Perfection peas. However, this virus differs from typical bean virus 2 in several respects. Symptoms on pea do not include definite or severe veinclearing which has been considered typical of the bean virus 2 group. The mottle produced in young apical pea leaves was more pronounced when compared to that incited by typical bean virus 2. Isolate 1 was inactivated when diluted 1:100,000 as compared to 1:1000 for other members of the bean yellow mosaic virus group, and it had a higher thermal inactivation point (60°–64° C.). It differed from the pod-distorting strain of bean virus 2, described by Grogan and Walker (2), in symptom expression and physical properties, and in having a much wider host range among legumes. The virus resembles Osborn's (8) pea virus 2 but differs from it in producing a less severe mottle, in being infectious to Great Northern U. of I. No. 1 bean, and in having less resistance to aging *in vitro*. Zaumeyer (14) in 1940 described pea mosaic virus 4 which resembles isolate 1 except for the different chlorotic mottle on peas and the yellow halolike leaf spotting which was present when pea mosaic virus 4 infected bean leaves. In contrast to isolate 1, it was infectious to alfalfa and not to Great Northern U. of I. No. 1 bean.

The virus described here does not fit into the pea common mosaic virus group (12) because no definite malformation of the leaves or stipules is incited, and plant stunt and apical foliage rosette, which are typical symptoms produced by members of the above virus group, are absent. Bean and soybean are not infected by the pea common mosaic virus, but were susceptible to isolate 1 from pea. The latter virus also differs in dilution tolerance and resistance to aging *in vitro* from those in the common pea mosaic group.

Isolate 1 differs from the pea enation virus (11) in that it does not produce enations on leaves or pods, nor does it induce malformation of the foliage and pods as does the enation mosaic virus. Pea enation virus, in contrast to isolate 1, infected Horal and Perfection peas, but did not infect bean and red clover. Physical properties also differ, especially tolerance to dilution, a tolerance of 1:2,000 but not 1:3,000 being reported for pea enation virus by Stubbs (11), compared to the 1:10,000 dilution tolerance of the pea virus isolate 1.

Differences between isolate 1 and the pea mottle virus described by Johnson (6) are numerous. Isolate 1 withstands aging only one day compared to 31 days for pea mottle virus. The isolate 1 virus is noninfective, and the pea mottle virus infective to the following hosts: Perfection pea, white clover, alfalfa, and cucumber. In further contrast, the pea mottle virus was not limited to the Leguminosae. Johnson did not find the mottle virus transmissible by the pea aphid.

The white clover viruses described by Pierce (10) and by Zaumeyer and Wade (15, 16) differ from isolate 1 in symptoms and physical properties, and they infect Wisconsin Early Sweet and Perfection peas, white clover, and alfalfa, but not soybean. Zaumeyer's (14) pea mosaic virus 5 and his alsike clover mosaic virus 2 are likewise dissimilar compared to the pea isolate 1. They do not infect red clover or soybean, and are infectious to alfalfa. They also give rise to different symptoms on pea—plant stunt, wilting, and death by pea virus 5, and necrosis and leaf drop by the alsike clover virus 2.

The comparisons cited above show that pea isolates 1, 2, 3, and 4 are strains of bean virus 2 but differ in some respects from the typical strain described by Pierce (9). They are distinct from other pea viruses described in the literature (12).

Particular significance may be attached to the host range studies made. All four isolates, in addition to affecting peas and beans, were capable of infecting red clover, yellow sweet clover, white sweet clover, and alsike clover. Red clover is very widely grown in Wisconsin and the sweet clovers are also used for pastures. These plants, and also alsike clover, can be found as volunteers along almost any road or highway in the State. Thus, an abundance of possible overwintering hosts are at hand and may very well account for the widespread occurrence of bean virus 2 in Wisconsin pea fields.

SUMMARY

The importance of bean virus 2 isolates as causal agents of pea mosaic in Wisconsin is shown.

Symptoms of four of these isolates on various hosts are described. Characteristic symptoms on pea include a mottle of the foliage, occasional very slight veinclearing, and little if any leaf wrinkle or dwarf. Plants in the greenhouse were not noticeably reduced in size. Symptoms in the field were more severe.

All four isolates were limited in host range to the Leguminosae, and identical in host range and symptoms. Alfalfa, Ladino white clover, and cowpea were not susceptible.

The relation of temperature to disease development study indicated that at 16° C. the incubation period of isolate 1 was greatly prolonged as compared to tests made at 20°, 24°, and 28° C. This isolate infected six of 18 pea varieties and all of the 12 bean varieties tested. It had a dilution end point of 1 : 100,000, resisted aging between 24 and 48 hr., and was thermally

inactivated between 60° and 64° C. It was easily transmitted by the pea aphid, both in controlled greenhouse tests and under natural field conditions. No transmission by pea or bean seed was observed.

Cross protection tests indicated similarity between the four isolates from pea and typical bean virus 2 in ability to reduce symptom severity of the pod-distorting strain on Michelite bean.

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LITERATURE CITED

1. DOOLITTLE, S. P., and F. R. JONES. The mosaic disease in the garden pea and other legumes. Phytopath. **15**: 763–772. 1925.
2. GROGAN, RAYMOND G., and J. C. WALKER. A pod-distorting strain of the yellow mosaic virus of bean. Jour. Agr. Res. [U.S.] **77**: 301–314. 1948.
3. HAGEDORN, D. J. Three viruses of canning pea. (Abstr.) Phytopath. **38**: 13. 1948.
4. ————, and J. C. WALKER. Cross protection tests between the Wisconsin pea streak virus and several strains of bean virus 2 from pea. (Abstr.) Phytopath. **39**: 11. 1949.
5. ————, and ————. Wisconsin pea stunt, a newly described disease. Jour. Agr. Res. [U.S.] **78**: 617–626. 1949.
6. JOHNSON, FOLKE. The complex nature of white-clover mosaic. Phytopath. **32**: 103–116. 1942.
7. JOHNSON, JAMES, and THEODORE J. GRANT. The properties of plant viruses from different host species. Phytopath. **22**: 741–757. 1932.
8. OSBORN, H. T. Studies on the transmission of pea virus 2 by aphids. Phytopath. **27**: 589–603. 1937.
9. PIERCE, WALTER H. Viroses of the bean. Phytopath. **24**: 87–115. 1934.
10. ————. The identification of certain viruses affecting leguminous plants. Jour. Agr. Res. [U.S.] **51**: 1017–1039. 1935.
11. STUBBS, MERL W. Certain viroses of the garden pea, *Pisum sativum*. Phytopath. **27**: 242–266. 1937.
12. WEISS, FREEMAN. Viruses described primarily on leguminous vegetable and forage crops. U. S. Dept. Agr., Pl. Dis. Reptr., Suppl. 154: 51–53. (Mimeographed.) 1945.
13. WHIPPLE, O. C., and J. C. WALKER. Strains of cucumber mosaic pathogenic on bean and pea. Jour. Agr. Res. [U.S.] **62**: 27–60. 1941.
14. ZAUMEYER, W. J. Three previously undescribed viruses of pea. Jour. Agr. Res. [U.S.] **60**: 433–452. 1940.
15. ————, and B. L. WADE. The relationship of certain legume mosaics to bean. Jour. Agr. Res. [U.S.] **51**: 715–749. 1935.
16. ————, and ————. Pea mosaic and its relation to the other legume mosaic viruses. Jour. Agr. Res. [U.S.] **53**: 161–185. 1936.

BORDEAUX MIXTURE FOR THE PREVENTION OF RUST AND CHOCOLATE SPOT OF BEANS

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Earlier work on the prevention of rust (*Uromyces fabae*) and chocolate spot (*Botrytis fabae*) of beans was reported by the writer for 1935–1937[1] and 1937–1939.[2] Spraying with Bordeaux mixture and, to a lesser extent, with Bouisol was found to be the most satisfactory method for controlling both diseases. The higher concentrations of Bordeaux mixture gave the most effective control.

Satisfactory results were always obtained by applying the initial spray when either disease was first detected, and then thoroughly protecting the plants until they were practically ripe. Spraying too early and too late had very little preventive effect.

The principal object of this work is to determine the economic value and the practical possibility of the use of Bordeaux mixture for rust and chocolate spot control in large fields of beans, and to devise an efficient and practical method for treating large areas of the crop.

Investigation was continued during the bean-growing seasons 1939–1942 in farms situated in the northern part of Lower Egypt, where both diseases cause great damage to the bean crop. A system of small drainage canals and ridges divides fields into units of about ½ to 3 feddans each (20 × 120 to 60 × 250 meters). During the spraying operations these drains, running parallel along the two long sides of each unit, facilitate the movement of the motor pump sprayer.

METHOD AND MATERIALS

The average spray application consisted of 1500 liters of 0.25 per cent Bordeaux mixture per feddan. For the 1 per cent Bordeaux mixture, 4 lb. copper sulphate and 4 lb. quicklime were added to 40 gal. water.

Small, 1½-HP hydraulic motor pump sprayers are practical because they can be carried easily and can readily be moved through the drains and along the sides of each unit of a field. Each machine can spray more than 3 feddans daily, using 1.5 gal. of petrol, 1 kg. of oil, and 1 kg. of grease per day. A hose about 20 to 40 meters long allows spraying half of the area of the unit while the motor is moving along the drain. Five men are needed to do the spraying and to carry the prepared dilute fungicide to the pump, five boys to carry the long hose so as to avoid breaking and killing the plants as much as possible, and six girls to carry the water for preparing the fungicide.

[1] El-Helaly, A. F. Preliminary studies on the control of bean rust. Min. Agr., Egypt, Bul. 201. 1939.

[2] El-Helaly, A. F. Further studies on the control of bean rust with some reference to the prevention of chocolate spot of beans. Min. Agr., Egypt, Bul. 236. 1939.

During the years 1939–1940, 1940–1941, and 1941–1942, respectively, the cost (in Egyptian £) per application per feddan was £0.330, £0.450, and £0.525; and the price of beans was £1.200, £1.620, and £3.500 the ardeb.

Application of Spray in Large Fields of Beans

During three growing seasons, 1940, 1941, and 1942, the dilute Bordeaux spray was applied to bean plots of variable size selected from commercial plantings on farms in the Delta region of Egypt.

In 1940 three sprays were applied, on January 14, February 3, and February 26, to two 1-feddan plots of the Baladi variety and to one 1-feddan plot of the Nobari variety grown on a farm at Zarka. Three adjacent plots of equivalent area were not sprayed and served as controls.

In 1941 three sprays were applied on January 16, February 10, and March 10 to three 1-feddan plots of Baladi beans on the farm at Zarka, and three adjacent plots were the controls. At El-Khilala six plots, each of ½ feddan, of Baladi beans were sprayed on January 24, February 24, and March 12; the six adjacent control plots were of the same size and were not sprayed. A single plot, approximately 2½ feddan, of the same variety grown at Meet El-Gharaka was sprayed twice, on January 21 and February 22.

Spray plots were selected on three farms at Kafr El-Baramone in 1942. The first farm provided a 3-feddan plot of Baladi beans to which the dilute Bordeaux was applied January 22, February 14, and March 3. On the second farm a 2-feddan plot of the Roomi variety was sprayed on January 25, February 15, and March 5. The third farm provided smaller plots, about 1¼ feddan each; two of the Baladi variety and one of the Nobari variety were sprayed January 24, February 16, and March 4. On each farm, control plots of the requisite area were adjacent to the spray plots.

TABLE 1.—*The rust and chocolate spot infections, the yields, and the net profit of varieties of bean sprayed with 0.25 per cent Bordeaux mixture*

Variety, locality, and year	Percentage of infection				Yield, in ardeb per feddan		Net profit, £
	Rust		Chocolate spot				
	Bordeaux	Control	Bordeaux	Control	Bordeaux	Control	
Baladi variety							
Zarka, 1940	15	90	15	75	9.38	4.28	5.11
Do 1941	10	90	10	90	5.73	2.47	4.02
El-Khilala, 1941	20	100	15	70	3.86	1.51	2.54
Meet El-Gharaka, 1941	15	70	15	50	6.40	4.21	2.71
Kafr El-Baramone, 1942	10	70	5	30	10.13	7.47	7.73
Do 1942	10	60	5	30	10.25	8.76	3.63
Nobari variety							
Zarka, 1940	10	80	10	60	6.64	2.87	3.53
Kafr El-Baramone, 1942	10	50	Tr	20	10.65	9.07	3.96
Roomi variety							
Kafr El-Baramone, 1942	Tr	25	Tr	10	7.33	6.25	2.21

The data in table 1 indicate that rust and chocolate spot diseases of beans can be controlled practically, effectively, and profitably in large fields. The resulting net profit per feddan varies from about £2.21 to £7.75 according to the condition of the crop, the incidence of the two diseases, the cost of spraying, and the price of beans.

The 1941 yield was not normal because 1) strong winds during flowering time caused the bean plants to shed many flowers and reduced yields on both the spray plots and the control plots, and 2) after heavy rains in early March many of the plants succumbed to root rots and the fruits were small and shriveled. In 1942, the differences between the yields of the treated plants and those of the untreated ones were small, because both rust and chocolate spot appeared late and were not severe.

DISCUSSION

Spraying with 0.25 per cent Bordeaux mixture has proved to be a satisfactory and practical method for controlling both rust and chocolate spot diseases in large areas of beans, even in years when one or both diseases are slight. A small sprayer, such as the 1½-HP hydraulic motor pump sprayer, is satisfactory because it can be moved easily through the drainage canals during the operation.

The application of such control, in treating large areas of the crop in the northern part of Lower Egypt, required no alteration in the method of preparation of the land for cultivation. Fields are already divided into small rectangular units of about ½ to 3 feddans each, their two long sides bordered by parallel drains usually about 20 to 60 meters apart. These drains facilitate movement of the motor pump sprayer along the two long sides of each unit.

Although the boys who carry the spraying hose may break some of the plants during the operation, the lower parts of these plants and in some cases the upper parts as well will escape severe damage and will probably bear fruits. Thus, the damage caused by the operation is not so great as might be supposed.

In the southern part of Lower Egypt and in Middle Egypt, where there is no such arrangement of units and drains, small, narrow, barren passages, about 50 to 70 meters apart, perform the same function as the drains in using the motor pump sprayer.

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